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Investigation of the rotational motion of self-assembled fatty acid films: An electron paramagnetic resonance line shape analysis

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We have investigated the electron paramagnetic resonance (EPR) of self-assembled stearic acid films adsorbed on an Al_2O_3-film. Doping the film with spin labels at different positions of the alkyl chain in order to make the films accessible for EPR spectroscopy provides an opportunity to investigate the rotational motion of the molecule along the alkyl chain. The temperature dependent EPR spectra show a strong dependence of the rotational motion of the molecules with variation of the location of the spin label along the chain. We study the rotational motion by means of the EPR line shape analysis. © *1998 American Institute of Physics.* [S0021-9606(98)00120-2]

I. INTRODUCTION

Over decades the line shape analysis of electron paramagnetic resonance (EPR) spectra has been established as a useful tool in order to characterize the dynamics of molecular motion. In the past line shape analysis has been frequently used in fields such as studies of biological membranes,[1,2] polymer science[3] or liquid crystals.[4] However, this method has only recently been established to study the rotational dynamics of molecules on single crystal surfaces.[5,6] Although a vast array of methods has been used to characterize various properties of adsorbates on single crystal surfaces there are still only a few methods that can provide information on the time scale between 10^{-6} and 10^{-10} s. Especially nuclear magnetic resonance (NMR) which has been very helpful in the investigation of molecular motion in liquid and solid phases is not suited to provide such information on molecules at monolayer coverage due to the lack of sensitivity (10^{17} spins)[7] whereas EPR spectroscopy due to its higher sensitivity (10^{12} spins) has been proven to provide information from submonolayer coverage.[5,8]

We present an EPR spectroscopic investigation of self-assembled fatty acid films adsorbed on an Al_2O_3 thin film substrate. Part of this investigation has been already published.[9] Since it is known from the literature that fatty acids form self-assembled monolayers on oxide surfaces[10,11] it is not surprising that it is possible to form the organic monolayer on a thin well ordered Al_2O_3 film as well. Due to the fact that the order of a self-assembled monolayer increases with increasing chain length we have used stearic acid ($C_{18}H_{36}O_2$) as a model substance. In order to make these films suitable for EPR spectroscopy it is necessary to introduce paramagnetic groups into the film. In particular we have used n-doxyl stearic acid (n-DXSA) where the paramagnetic group, an oxazolidinyl ring, may be positioned at different locations within the hydrocarbon chain. Such spin labels have been used as paramagnetic probes, for example, in the study of natural and synthetic membranes,[12] and it will be interesting to compare our results with those studies.

II. EXPERIMENT

The EPR measurements were performed with a conventional EPR spectrometer (Bruker B-ER 420) that has been improved by building in a X-band microwave bridge (Bruker ECS 041 XK) and a lock-in amplifier (Bruker ER 023 M). This modification led to a considerable increase in the signal-to-noise ratio. This EPR spectrometer was adapted to a specially designed ultrahigh vacuum chamber that has been described in detail previously.[13] In order to characterize the substrate the ultrahigh vacuum chamber was equipped with a quadrupole mass spectrometer as well as a combined LEED/Auger unit.

The Al_2O_3-film was prepared on a NiAl(110) single crystal according to the recipe by Jaeger *et al.*[14] First, the substrate was cleaned by subsequent cycles of sputtering with Ar ions and annealing to 1000 °C. Afterwards the crystal was oxidized and the amorphous oxide film was annealed at elevated temperatures in order to build a well ordered, dense Al_2O_3-film.

The doxyl stearic acids as well as the pure stearic acid were purchased (Sigma, Munich) and used without further purification. The fatty acid films were prepared by immersion of the Al_2O_3 substrate into a mixture of n-doxyl stearic acid and stearic acid in toluene. The temperature of the solution was room temperature in the case of the 5-doxyl stearic acid films and 28 °C in the case of the 12- and 16-doxyl stearic acid films until it is stated otherwise in the text. The overall concentration of the molecules in the solution was always 1 mM and the molar fraction of each component is given in the text. The immersion times were varied between 24 and 100 h without an effect on the resulting EPR spectra. The sample was removed from the solution, rinsed several times with toluene and subsequently with ethanol and water and dried in air. The freshly prepared films were transferred into the vacuum chamber. The EPR spectra were recorded under high vacuum conditions with a pressure lower than 10^{-7} mbar. Under these conditions the self-assembled films are stable for weeks.

The program used to simulate the line shape of the EPR spectra was basically written by Beckendorf.[15] The descrip-

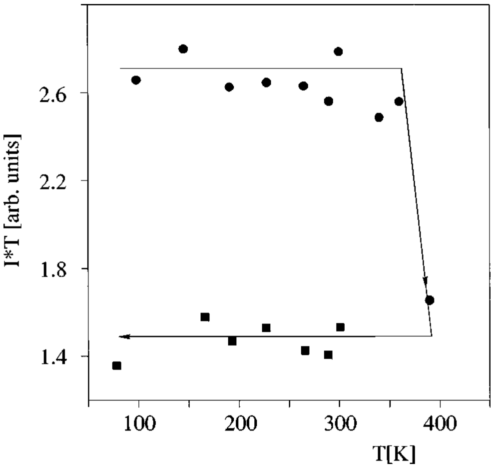

FIG. 1. Curie corrected intensity of a 5-doxyl stearic acid film versus temperature.

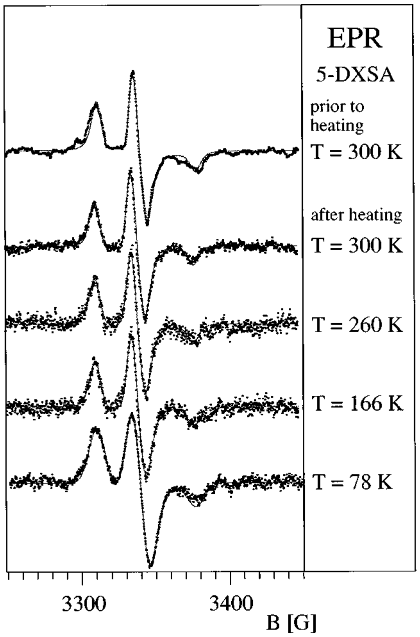

FIG. 2. Temperature dependent EPR spectra of a 5-doxyl stearic acid film after heating to 400 K and then cooling to the given temperature. Points represent the measured spectra, full lines the corresponding simulations. The spectra are normalized to a constant peak-to-peak height.

tion of the molecular motion is based on a program package developed by Freed and co-workers,[16] which utilizes the solution of the stochastic Liouville equation for the description of dynamic processes.[17] The molecular constants, namely the g- and A-tensor components, necessary to perform the computer simulations have been determined from studies of the films at low temperatures that have been described elsewhere.[18] All spectra are carefully background subtracted in order to eliminate a background signal which is caused by the ferromagnetic resonance of small nickel clusters dissolved in the NiAl single crystal due to the preparation procedure.[19]

III. RESULTS AND DISCUSSION

A. Temperature stability of the films

The discussion of the dynamic properties of the self-assembled film requires the knowledge of the stability of the film with respect to a change in sample temperature in order to rule out changes in the line shape of the spectra that are caused by irreversible structural changes. Figure 1 shows the integral intensity of a 5-doxyl stearic acid film with respect to the temperature of the film. The molar fraction of the labeled molecules in solution was 8% which is sufficiently low to avoid spin–spin interactions.[18] At the ordinate the product of intensity and temperature rather than the intensity itself is plotted to directly account for Curie's law. Raising the temperature up to 365 K no significant deviation from the predicted behavior in the Curie corrected intensity could be observed. However, increasing the temperature above 370 K leads to a rapid decrease of the intensity. Upon decreasing the temperature of the film again the system behaves according to Curie's law again.

There are two different mechanisms which may give rise to the observed behavior. On the one hand it is possible that the spin label decomposes at the higher temperatures and hence leads to the loss of the EPR intensity. It is well known that under atmospheric conditions the stability of the paramagnetic center is strongly reduced with increasing temperature.[20] However, with respect to the experimental conditions ($p < 10^{-7}$ mbar) the number of reactive partners is considerably reduced. On the other hand desorption of molecules from the surface may cause the loss of signal intensity.

The desorption of molecules from the surface should change the structure of the self-assembled film noticeably. Vacancies are created and therefore the free volume of the film and the mobility of the molecules should increase considerably. The EPR spectra of a 5-doxyl stearic acid film heated up to 400 K together with a spectrum of the film prior to the heating are shown in Fig. 2. Comparing the spectrum at 300 K with the spectrum of the undisturbed film at the same temperature a noticeable change in the line shape occurs. The spectrum after the heating procedure shown here cannot be described by assuming an ensemble of rigid molecules as it was possible for the freshly prepared film.[18] Although the effect is not very large the spectrum exhibits a significantly smaller A_z-splitting than the film before heating. This reduction of the A_z-splitting is caused by rotational motion of the spin labels on the time scale of the experiment. The simulation of the line shape gives a rotational constant of 3×10^6 Hz. The description of the spectra according to the assumption of rigid molecules is possible below 270 K. Compared to the undisturbed film the onset temperature for the rotational motion drops by almost 50 K which is a considerable decrease of the rotational energy connected with the rotational motion. In anticipation of the discussion of the rotational motion in the next section it should be mentioned that the changes in line shape that are attributed to rotational motion of the molecules differs considerably between the freshly prepared and the heated film. Whereas the disturbed system exhibits a decrease of the A_z-splitting from the very

TABLE I. g- and A–tensor components for the different n-doxyl stearic acids.

	g_x	g_y	g_z	A_x[G]	A_y[G]	A_z[G]
From Ref. 31	2.0088	2.0061	2.0027	6.3	5.8	33.6
5-DXSA	2.0089	2.0062	2.0027	6.4	5.9	33.5
12-DXSA	2.0089	2.0063	2.0027	6.1	5.6	32.7
16-DXSA	2.0088	2.0059	2.0027	6.0	5.7	33.8

beginning this splitting remains unchanged in the case of the undisturbed film.

As the additional free volume of the molecule influences the rotational behavior of the spin label the same should hold true for the alkyl chain. As has been discussed in detail in a previous publication[18] the rotational motion of the methylene groups directly connected to the spin label can be monitored by means of a Gaussian broadening of the spectra with decreasing temperatures. The broadening is caused by unresolved proton hyperfine interactions which increase in magnitude with decreasing rotational motion of the methylene groups. As the free volume of the structure is increased one should expect that the rotational motion of the methylene groups are less hindered by the environment and therefore it should freeze at lower temperatures. Whereas the undisturbed film reveals a broadening of the lines below 180 K the line width of the heated sample increases below 166 K. For the spectrum at 78 K shown at the bottom of Fig. 2 the motion of the methylene groups are frozen again and the line shape of the spectrum is comparable to the one of the unperturbed film.

Referring to the two mechanisms proposed above, namely the decomposition of the spin label and the desorption of molecules we can conclude that the desorption of molecules is more likely than the decomposition of the spin label, because of the large effect caused by the heating procedure which is connected to a distinct increase of free volume in the film.

B. The dynamic behavior of the films

In the following sections we want to discuss the dynamic behavior of the self-assembled films by means of the change in the line shape of the EPR spectra. More precisely, we want to discuss the change of the line shape which is due to the motion of the spin label on the time scale of the EPR experiment. In the case of rigid molecules on the time scale of the EPR experiment the line positions are determined by the g- and A-tensor components and the Euler angles between the molecular principal axis and the laboratory coordinate system. The EPR spectra can be obtained by integrating over all molecular orientations of the ensemble. If the molecules, however, rotate very rapidly on the time scale of the experiment the anisotropy of the resonance positions in space vanishes due to the fast rotation. Therefore the spectrum is determined by an isotropic g-factor and the isotropic hyperfine interaction. For the radical used in this investigation with the unpaired electron located at a ^{14}N-nucleus with a nuclear spin $I=1$ the fast rotation leads to the well known triplet spectrum centered at the isotropic g-factor and split by the isotropic hyperfine interaction.

The temperature range for the three different films investigated here, namely 5-, 12-, and 16-doxyl stearic acid films, showing changes in the line shape due to rotational motion of the spin label increases drastically while increasing the distance between the spin label and the substrate.[18] Hence we start the discussion of the rotational motion with the 16-doxyl stearic acid films as these films reveal the greatest effects. In order to discuss the dynamic changes of the line shape it is necessary to know the g- and A-tensor components of the molecules. These constants must be determined in the rigid limit case in order to avoid changes of the values due to dynamic effects. Table I summarizes the results of the low temperature investigations presented elsewhere.[18]

1. Dynamic properties of 16-doxyl stearic acid

Investigations of 16-doxyl stearic acid films at low temperatures have shown that the spin labels become rigid on the time scale of the EPR spectroscopy below 190 K.[18] Therefore the temperature range investigated here is restricted to temperatures above 190 K. Figure 3 shows the spectra of two 16-doxyl stearic acid films. The spectra 215, 260, and 300 K originate from a film with a molar fraction of labeled molecules of 10% whereas the spectra at higher temperatures stem from a film with 6% 16-doxyl stearic acid in

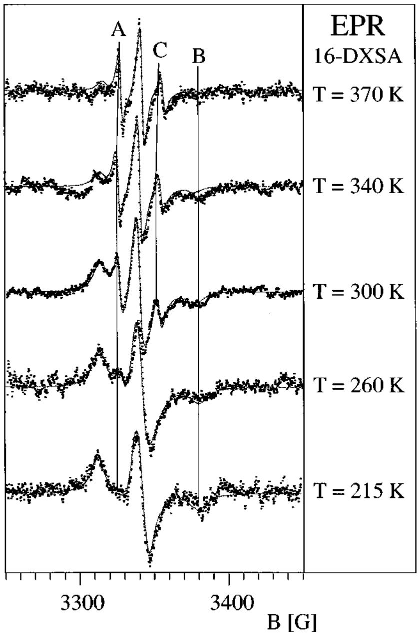

FIG. 3. Temperature dependent EPR spectra of 16-doxyl stearic acid films between 215 and 370 K. Points represent the measured spectra, full lines the corresponding simulations. The spectra are normalized to a constant peak-to-peak height.

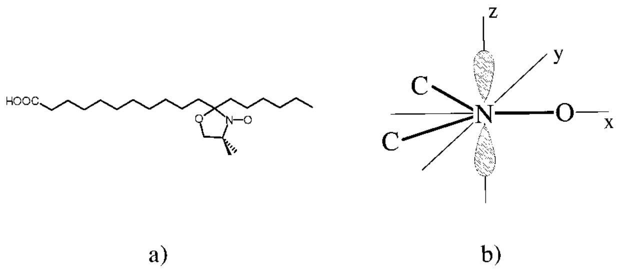

FIG. 4. (a) Schematic representation of a 12-doxyl stearic acid molecule, (b) principal axis of the g- and A-tensors for the nitroxides.

the solution. Looking at the spectrum at 215 K no drastic change of the line shape occurs compared to the spectra of the rigid spin labels. However, the region between the two maxima (labeled with A) drops slightly towards higher field whereas the rigid limit spectrum shows a plateau in this region. Looking at the other spectra of the temperature series the change in the line shape becomes more clear. While increasing the temperature a new feature appears in this region (A). The decrease of the spectrum at 215 K can be interpreted as a redistribution of spectral intensity towards the region typical for rotationally excited molecules. It is worthwhile to notice that the A_z-splitting of the hyperfine constant remains unchanged for these temperatures. Beside the small peak at 3325 G, the spectrum at 260 K exhibits changes in the high field region around 3380 G (B). The minimum of the spectrum becomes broader compared to the spectra at lower temperatures. This is also an indication for a shift of spectral intensity towards the center of the spectrum. The line shape of the spectrum changes more drastically when the temperature is increased to 300 K. The feature at 3325 G has then developed into a sharp, distinct peak. Furthermore, the signal with a typical triplet structure expected for rotationally excited molecules occurs at 3355 G (C) for the first time at this temperature. Further increase of the temperature above 300 K emphasizes the dynamic features of the spectra. This can be checked for several aspects. First, the relative intensity of the high field peak of the triplet becomes sharper compared to the low field pendant. This implies that the relative peak intensities of these two features become similar as it would be expected for very rapid isotropic rotation of the molecules. Second, the line width of the triplet structure decreases considerably, and third the spectral intensity located at the position of the rigid limit spectrum is shifted toward the center of the spectrum and leads to a decrease of the effective A_z-splitting.

2. Theoretical description of the motion

The theoretical description of the line shape has to face the problem that the line shape of the EPR spectra exhibits simultaneously features connected with fast as well as slow rotational motion of the spin labels. The program used to describe the rotational motion of the spin labels assumes that this motion can be characterized by two effective rotational constants, namely a rotational axis along the x-axis of the tensorial framework and an axis perpendicular to this axis. The orientation of the g- and A-tensor framework with respect to the molecular structure is shown in Fig. 4. Although the applied model based on the work described by Freed *et al.*[16,17] offers several different models to simulate the motion of the molecules it was impossible to describe the changes in the line shape of the spectra in a temperature series with one single consistent set of model parameters. The failure of this method may reflect the fact that the models used to simulate the line shape oversimplify the complexity of the motional behavior of the self-assembled film. Similar complex changes in the line shape have been reported in the literature for other systems such as liquid crystals or protein/lipid mixtures.[1,21]

On a microscopic scale a different mechanism may be responsible for the rotation of the spin label. From Fig. 4 it is evident that a rotation around the C–C bond between the carbon atoms 11 and 12 leads to a rotation of the spin label. However, a single rotation around this bond would also imply that the rest of the alkyl chain rotates in space which is highly unlikely in densely packed systems for simple geometrical reasons. Historically, Schatzki has suggested the first idea to overcome this problem.[22] He suggested a so-called crankshaft motion where two conformational motions occur close in time and space so that the initial and final position of the tail are identical. There are, however, several arguments against this kind of compensating transition which has been discussed in detail in the literature.[23,24] With respect to the rates of the conformational reorientation of the chains it has been shown theoretically as well as experimentally that the overall mobility of the segments increases with increasing distance from the headgroup.[24,25] This is due to the fact that the outer segments of the chain profit from the mobility of inner segments which is a direct consequence of the frictional forces within an assembly. It is clear from this discussion that the mobility of a segment of the chain depends on the one hand on the mobility of the rest of the chain and on the other hand on the environment of the chain, e.g., the density of the surrounding molecules or the dynamics of these molecules. Besides these internal motions of the chain also collective fluctuations within the whole system or other motional processes that could induce reorientation of the chain axis can occur which can hardly be explained on the basis of single microscopic excitations. As the EPR line shape reflects the overall motion of the whole ensemble of spin labels it is reasonable that the line shape cannot be described by two effective rotational constants.

In order to overcome the restriction of two effective rotational constants we have tried to superimpose components with different rotational constants. The superposition of several components increases the flexibility of the fitting procedure considerably, however, the number of independent parameters exceeds reasonable limits very rapidly. Although the g- and A-tensor components have been determined independently at low temperatures,[18] the rotational constants, the line widths, necessary potential parameters, as well as the relative weights of the components remain as free parameters. In order to reduce this large number of parameters we tried to define some fixed rotational modes and only change the relative weights of these components throughout the simulation of a temperature series. Since the g- and

A-tensors of the doxyl spin label are almost axially symmetric, only one rotational constant influences the line shape of the spectrum dominantly. Therefore we have always chosen the two rotational constants to be the same within one component. It should be noticed, however, that these motional modes are not simply related to specific single molecular rotational states. These modes should be interpreted as a combination of several molecular rotational modes within a certain rotational time scale. This implies that there is no unique criterion of how to choose the components of the fit. The parameters used for the different components are therefore determined iteratively for the different spectra of the temperature series. We used altogether four components, however, only three of them are used for a single spectrum.

Since the spectra at 215 and 260 K exhibit intensities in the region of the rigid limit spectra we have chosen the spectrum at the rigid limit as the first component of the fit. This component covers the rotational motion below approximately 10^6 Hz. The next component should account for the features connected with the fast rotational motion of the spin labels, namely, the triplet structure that occurs in the spectra above 300 K. If the rotational motion of the spin labels would be isotropic in space the splitting of the lines in the triplet should be equal to the isotropic hyperfine tensor $[a_{iso}=1/3(A_x+A_y+A_z)]$. The determination of the peak splitting shows that it is about 1.5 G lower than expected with respect to the isotropic value. This observation indicates that the motion of the spin label is not fully isotropic in space. To account for this anisotropy we introduce an additional potential along a certain direction in space. The director of the potential was chosen along a principal axis of the g- and A-tensor since there is no experimental evidence for introducing a tilt angle between the principal axis of the tensors and the director of the potential in the simulation. The introduction of a potential along a certain axis leads to a shift of the maxima of the triplet towards the rigid limit value of the affiliated tensor component. Consequently, the potential should be oriented along the x- or the y-axis since the measured spectra exhibit a smaller splitting compared to the isotropic value. This is in variance with investigations of liquid crystals as well as model membrane systems where the potential is often along the z-axis of the spin label.[26,27] The best fit is obtained if the potential is directed along the y-axis. The adjustment of the rotational constant for the spectrum at 300 K shows that a value of 10^8 Hz is appropriate to describe the differences in linewidth and intensity of the hyperfine triplet. Between the two components, i.e., the rigid limit and the rotational state with a rotational constant of 10^8 Hz there is a gap of two orders of magnitude in the rotational constant. Especially the small changes of the line shape in the spectra at 215 and 260 K cannot be described properly with these two components alone. Therefore we introduce a third component with a rotational constant of 1.2×10^7 Hz which has been found to describe best the small changes in the line shape. In analogy to the rotational behavior at higher temperatures, namely the shift of the hyperfine splitting with respect to the isotropic value, one should expect that the rotational motion at lower temperatures is also anisotropic in space. The simulation of the spectra at 215 and 260 K shows that the assumption of an ordering potential is indeed crucial to simulate these two spectra within a consistent set of parameters.

TABLE II. Parameters used for the simulation of the spectra of the 16-doxyl stearic acid films shown in Fig. 3. D_{xy} and D_{zz} denote the rotational constants. λ_{0y}^2 represents the parameter of the ordering potential (Ref. 16).

T [K]	λ_{0y}^2	ΔB[G]	rel. int. [%]
Component of rigid spin label			
215	—	8.5	48
260	—	8.3	39
300	—	8.1	18
Component with $D_{xy}=D_{zz}=1.2\times10^7$ Hz			
215	0.45	6.5	52
260	0.45	6.4	61
300	0.45	6.1	74
340	0.45	6.0	73
370	0.2	4.1	60
Component with $D_{xy}=D_{zz}=1\times10^8$ Hz			
300	0.65	4.8	8
340	0.65	3.3	22
370	0.1	2.9	27
Component with $D_{xy}=D_{zz}=8\times10^8$ Hz			
340	—	3.0	5
370	—	1.9	13

The change in the line shape while increasing the temperature above 300 K indicates a further increase of the rotational frequency of motion. Especially the gain of intensity of the high field component of the triplet cannot be described properly within the presented scheme. Therefore we introduce a fourth component with a rotational constant of 8×10^8 Hz. On the other hand, the intensity at the positions of the rigid limit spectrum vanishes, so that the rigid limit component is no longer necessary for the simulation of the high temperature spectra. Furthermore, the analysis of the high temperature spectra reveals that the triplet splitting increases with increasing temperature which indicates that the motion of the spin labels becomes more isotropic with increasing temperature. For the spectrum at 370 K the triplet splitting is nearly equal to the isotropic value. The parameters used to simulate the spectra shown above are summarized in Table II.

In order to describe the EPR spectra of the temperature series we have adjusted the relative weights of the components for each spectrum. Figure 5 shows the change of the relative weights for the spectra in Fig. 3. For the low temperature spectra (215, 260 K) the line shape can be described by the two slow components. With increasing temperature the fraction of the rigid limit spectrum decreases monotonically and vanishes above 300 K. Compared to that the component with a rotational constant of 1.2×10^7 Hz increases in intensity up to 315 K and decreases afterwards. At 300 K the component with the rotational constant of 10^8 Hz appears and gains intensity with increasing temperature. For the two

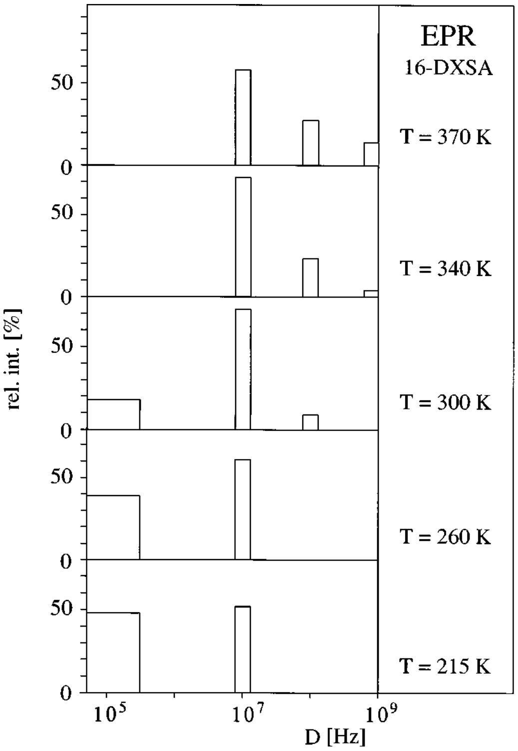

FIG. 5. Relative population of the components used to simulate the spectra of the 16-doxyl stearic acid film shown in Fig. 3.

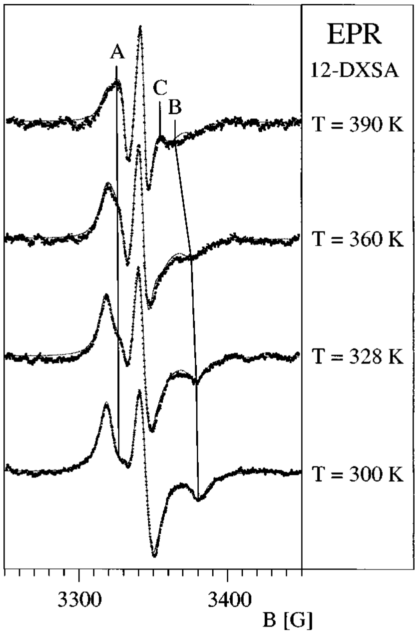

FIG. 6. Temperature dependent EPR spectra of 12-doxyl stearic acid films between 300 and 390 K. Points represent the measured spectra, full lines the corresponding simulations. The spectra are normalized to a constant peak-to-peak height.

spectra at highest temperature the component with highest rotational frequency has to be taken into account and it gains intensity with increasing temperature.

Except for the spectrum at 340 K, which shows a rather poor correspondence in the extreme regions of the spectrum, the line shape of the spectra can be described satisfactory within this model. However, the problems in the spectrum at 340 K are due to a poor background subtraction. The relative populations of the different components with temperature are shown in Fig. 5 and correspond to a behavior expected for Boltzmann distributions. For increasing temperature the mean value of the rotational constant shifts towards higher rotational frequencies and simultaneously the distribution of the rotational constants becomes broader.

It is, however, worthwhile to comment on the parameters used to fit the spectra. The primary idea of this model was to simulate the line shape with several fixed so-called ''rotational modes'' and to look for the evolution of the relative weights of the components. As can be seen in Table II this could not be achieved throughout the temperature series without adjusting the linewidths of the spectra. The decrease in linewidths with increasing temperature is expected due to an increase of the rotational excitement of the molecules, e.g., through the decrease of dipolar interaction.[28] The rotational model used to describe the line shape must take this effect into account. This means that the molecular rotational states comprising the several dynamic components of our model change with the temperature. Looking a little bit closer at the parameters it becomes clear that the largest changes in the parameters occur at the highest temperature, namely 370 K. As has been discussed in the previous section, in this temperature range irreversible changes in the structure of the system occur so that it is unlikely that the model adjusted for the undisturbed system is fully suitable to describe the perturbed system properly. On the basis of these more general considerations we are prepared to discuss the remaining systems separately.

3. Dynamic properties of 12-doxyl stearic acid

Figure 6 shows the change in the line shape of a 12-doxyl stearic acid film in the temperature range between 300 and 390 K. The first indications of slow rotational motion can be observed slightly below 300 K. The principal changes in the line shape are similar to those of the 16-doxyl stearic acid, although the linewidths of the spectra are significantly higher than in the other two systems due to a higher concentration of the labeled molecules (15%). First, the spectrum shows a drop in intensity between the two maxima at 3315 and 3330 G. A subsequent increase of the temperature leads to the formation of an additional feature at approximately 3325 G (A). Simultaneously, the high field minimum (B) becomes broader and shifts towards the center of the spectrum so that the spectrum loses structure until the additional peak (C) corresponding to the triplet structure appears at 390 K. However, the spectra of the 12-doxyl stearic acid film shown here reveal an important difference compared to the other two systems investigated. Whereas the spectra of the 16- as well as the 5-doxyl (see below) stearic acid film exhibit intensity in the region of the rigid limit spectrum, although the spectra contain characteristics of rotationally excited molecules as well, the spectra of the 12-doxyl stearic acid reveal a shift of the A_z-component towards the center of the spectrum from the very beginning (we comment on this in the next section). With respect to the model suggested

TABLE III. Parameters used for the simulation of the spectra of 12-doxyl stearic acid films shown in Fig. 6. D_{xy} and D_{zz} denote the rotational constants. λ_{0y}^2 represents the parameter of the ordering potential (Ref. 16).

	Component with $D_{xy}=D_{zz}=5\times10^6$ Hz		
T [K]	λ_{0y}^2	ΔB[G]	rel. int. [%]
285	0.1	11.2	100
300	0.1	11.0	95
328	0.1	10.5	83
360	0.1	9.8	62
390	0.1	8.4	53
	Component with $D_{xy}=D_{zz}=3\times10^7$ Hz		
T [K]	λ_{0y}^2	ΔB[G]	rel. int. [%]
300	0.15	8.6	5
328	0.15	8.0	17
360	0.15	6.5	25
390	0.15	5.6	27
	Component with $D_{xy}=D_{zz}=1\times10^8$ Hz		
T [K]	λ_{0y}^2	ΔB[G]	rel. int. [%]
360	—	4.8	13
390	—	4.6	20

above this implies that the rigid limit component is no longer necessary for the simulation of the spectra. Therefore we have replaced the rigid limit component by a component with a rotational constant of 5×10^6 Hz. This component was adjusted to represent the A_z-splitting of the spectrum at 300 K correctly. Besides this component two further components with a rotational constant of 3×10^7 and 10^8 Hz were used in order to describe the effects of the faster rotations. Following the model described above we also introduced an ordering potential. However, the potential is significantly weaker than in the case of the 16-doxyl stearic acid film. It is also possible to simulate the spectra without an ordering potential although the description of the sharp descent of the spectrum between 3325 and 3330 G can be improved by introducing a small potential. The parameter sets used to simulate the spectra are summarized in Table III.

The relative populations of the components used to simulate the spectra of Fig. 6 are shown in Fig. 7. As in the case of the 16-doxyl stearic acid the relative weight of the components with higher rotational frequencies increases with increasing temperature. Whereas the spectra at 300 and 328 K are mainly determined by the two slow-motion modes, the components with the higher rotational frequency gains intensity for the two spectra at elevated temperatures. In analogy to the 16-doxyl stearic acid films we had to adjust the parameters of the model especially for the spectrum at 390 K. At this temperature the structure of the film has changed considerably as has been shown in Sec. III A. And the change of the parameters may also be attributed to the change in the structure of the film.

4. Dynamic properties of 5-doxyl stearic acid

The shift of the spin label towards the surface increases the onset temperature of the rotational motion on the time scale of the EPR experiments again to higher temperatures. For the 5-doxyl stearic acid film discussed here the spectrum at room temperature can be described as a rigid limit spectrum as far as the spin label is concerned. In order to provoke changes in the line shape that are connected to rotational motion of the spin labels the temperature has to be increased to 325 K. Figure 8 shows the EPR spectra of a 5-doxyl stearic acid film between 325 and 385 K. As it was already

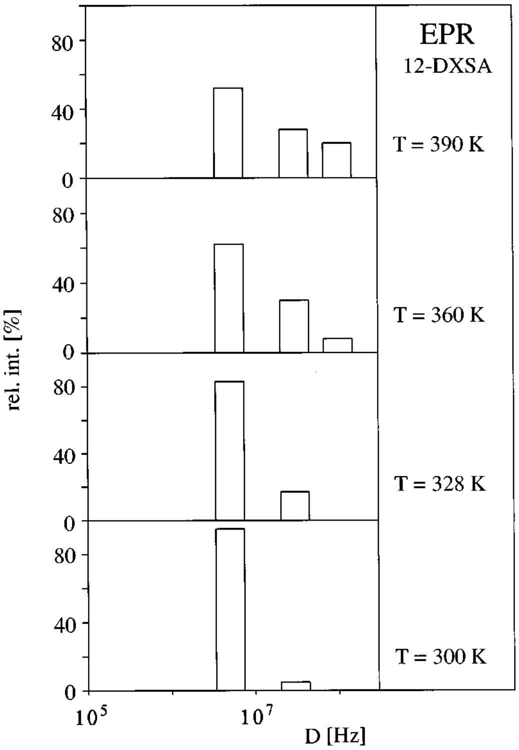

FIG. 7. Relative population of the components used to simulate the spectra of the 12-doxyl stearic acid film shown in Fig. 6.

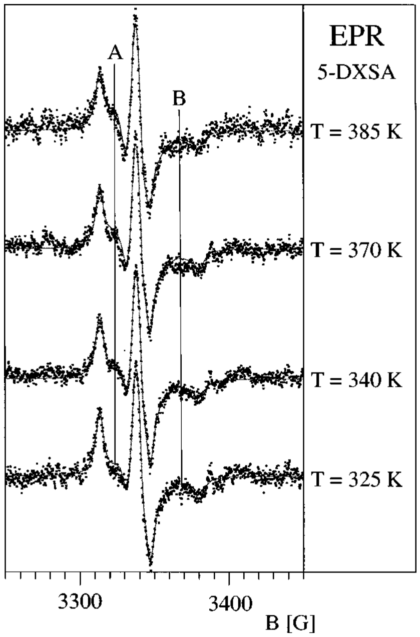

FIG. 8. Temperature dependent EPR spectra of 5-doxyl stearic acid films between 325 and 385 K. Points represent the measured spectra, full lines the corresponding simulations. The spectra are normalized to a constant peak-to-peak height.

TABLE IV. Parameters used for the simulation of the spectra of 5-doxyl stearic acid films shown in Fig. 8. D_{xy} and D_{zz} denote the rotational constants. λ_{0y}^{2} represents the parameter of the ordering potential (Ref. 16).

	Component of rigid spin label		
T [K]	λ_{0y}^{2}	ΔB[G]	rel. int. [%]
325	—	7.1	80
340	—	7.0	73
370	—	6.5	65
385	—	5.85	58

	Component with $D_{xy}=D_{zz}=2\times10^{7}$ Hz		
T [K]	λ_{0y}^{2}	ΔB[G]	rel. int. [%]
325	0.5	6.8	20
340	0.5	6.8	27
370	0.5	6.2	35
385	0.5	5.6	42

described for the 16-doxyl stearic acid film the first indication of molecular motion is the decrease of the spectrum in the region between the two maxima (A). While increasing the temperature the high field site of the maximum at 3310 G gains intensity and we observe the formation of a shoulder at the high field side of the peak at 3310 G. The changes in the high field part of the spectra are less pronounced. However, the redistribution of spectral intensity to the center of the spectrum can be observed through the broadening of the high field extremum and the corresponding decrease of the maximum at 3365 G (B). In analogy to the 16-doxyl stearic acid film the spectra reveal intensity at the positions of the rigid limit while dynamic features are present in the spectrum.

We have used the same model as discussed in Sec. III B 2 for the simulation of the spectra shown as full lines in Fig. 8. In order to describe these spectra it was, however, sufficient to superimpose only two components. Besides a static component to account for the intensity at the extreme positions we choose a dynamic component with a rotational constant of 2×10^{7} Hz. The ordering potential used for the dynamic component was adjusted for the spectra at 340 and 370 K. Although the spectrum at 325 K could be described without any potential the introduction of the potential improves the simulation of the spectra at 340 and 370 K considerably. Especially the description of the descent at 3325 G can be improved by assuming an ordering potential. The direction of the potential is again oriented along the y-axis of the g- and A-tensors. A summary of the parameters used to simulate the spectra is presented in Table IV.

Figure 9 exhibits the relative populations of the two components with respect to the temperature. The static component of the fit decreases monotonically with increasing temperature to approximately 60% at 385 K. This large static fraction and the low rotation frequency of the dynamic component show that the dynamic aspect in this system is much less pronounced compared to the two other systems already discussed. Finally, it should be mentioned that the linewidth of the simulation shows the same effect as was discussed in the previous systems, namely, a decrease of the linewidth for the high temperature spectra where irreversible change of the structure occurs.

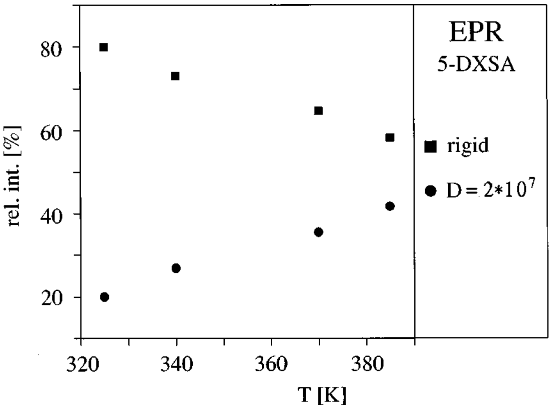

FIG. 9. Relative population of the components used to simulate the spectra of the 5-doxyl stearic acid film shown in Fig. 8.

5. Comparison of the dynamic behavior of the three systems

The behavior of the three systems can be summarized as follows:

(1) The onset temperature for rotational motion of the time scale of the EPR experiments decreases with increasing distance of the spin label from the surface.

(2) The line shape of the spectra can be described by superposition of spectra with different ''rotational constants.''

(3) The fraction of components with higher ''rotational constants'' increases with increasing temperature.

(4) For a given temperature the rotational motion of the molecules increases drastically with increasing distance to the surface.

(5) The rotational motion of the molecules is not isotropic in space.

Besides these general statements there are also differences in the behavior of the three systems. The most important one exists between the 12-doxyl stearic acid and the other two systems. Whereas the rotational motion of the 12-doxyl stearic acid film leads to a decrease of the A_z-splitting from the very beginning the splitting remains fixed at the beginning for the other two systems. Furthermore, the ordering potential used to describe the line shape of the spectra properly is less pronounced for the 12-doxyl case compared to the others. This is in agreement with the line shape analysis of the low temperature regime where we also find considerable differences between the 12-doxyl stearic acid film and the two other systems.

The change of the A_z-splitting as well as the decrease of the potential can be explained by a more isotropic rotation of the molecules. This is due to the fact that the order, and through this the density of the systems, decreases with increasing concentration of the spin labels in the film. Experimental evidence for the reduction of the order of the film with increasing concentration of the spin labels is the strong decrease of the linear dichroism in the near edge x-ray absorption fine structure (NEXAFS) spectra when the concentration of the spin labels is increased.[18] Additional evidence for a strong dependence of the rotational behavior of the self-assembled film on the structure are the large changes of

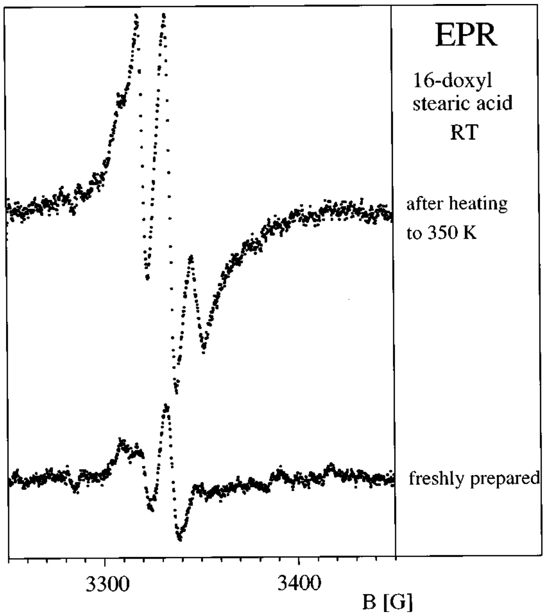

FIG. 10. EPR spectra of a 16-doxyl stearic acid film prepared below 25 °C. Bottom: freshly prepared; top: after heating to 350 K.

the parameters needed to describe the spectra at highest temperature where thermal decomposition of the systems occurs.

C. Temperature dependent adsorption behavior

The reversible temperature dependent changes in the line shape of 12- and 16-doxyl stearic acid as discussed in the previous sections were observed as long as the temperature of the solution used for the preparation of the film exceeded 25 °C. However, decreasing the temperature of the solution below 25 °C leads to significant changes. The lower spectrum in Fig. 10 shows a 16-doxyl stearic acid film prepared below 25 °C with a molar fraction of the labeled molecules of 8%. Although the molar fraction of the labeled molecules was similar to the preparation used for the film discussed in the last section (see Fig. 3) the linewidth of the film is increased. Heating of the film prepared below 25 °C outside the vacuum chamber to temperatures above room temperature, after mounting it into the vacuum chamber, changes the line shape as well as the linewidths in the spectrum considerably. The upper spectrum in Fig. 10 exhibits a spectrum measured at room temperature after heating the film to 350 K. In order to elucidate the difference between the two spectra both are plotted as measured on the same scale. It is clear that not only the linewidths and the line shapes but also the intensity of the spectrum changes significantly. The behavior of the integral intensity for a temperature cycle from 55 to 370 K and back to 75 K is plotted in Fig. 11. In order to account for Curie's law the product of temperature and intensity ($I \cdot T$) is plotted on the ordinate (see Fig. 1). The Curie corrected intensity is almost constant while heating the sample from 55 K to room temperature. An additional increase of the temperature by 15 K enhances the intensity by a factor of 3. A subsequent increase of the temperature up to 370 K increases the intensity further, however, the gain in intensity is less pronounced. The spectra taken while cooling the film back down to 75 K again exhibit significant deviations from Curie's law. This is in contrast to the temperature induced destruction of the film discussed in Sec. III A where the film behaves according to Curie's law while decreasing the temperature (see Fig. 1). The decrease of the intensity with decreasing temperature is not reversible as indeed shown by the intensities and linewidths before and after the heating procedure.

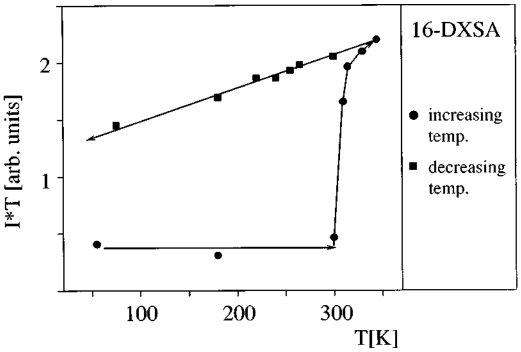

FIG. 11. Curie corrected intensity of a 16-doxyl stearic acid film adsorbed below 25 °C for a temperature cycle from 55 K to 370 K and back to 75 K.

In order to find an explanation for this behavior one should keep in mind that the doxyl stearic acid molecules have two hydrophilic groups that are able to adsorb on the aluminum oxide surface, namely the carboxyl group and the spin label itself. Investigations of the adsorption behavior of the stable radical di-tert-butyl nitroxide (DTBN) on the same aluminum oxide surface showed strong chemisorption of the NO group on the surface.[29] Due to the similarity between the DTBN molecule and the spin label used in these experiments we may conclude that the spin labels as well as the carboxyl group adsorb on the aluminum oxide film. However, the adsorption of the spin label is hindered compared to the DTBN molecule as the rigidity of the heterocyclic ring and the steric restrictions of the long alkyl chain decreases the accessibility of the NO group for the surface. The investigation of the adsorption behavior of DTBN on the Al_2O_3-film has shown that a majority of the molecules adsorbed on the surface are EPR inactive. The loss of the EPR signal is due to a specific adsorption mechanism of the molecules.[29]

If we assume that the molecules adsorb as a bidentate, i.e., with both functional groups attached to the surface, this would result in loop structures within the film. A schematic representation of such a structure is shown in Fig. 12. Evidence for the formation of similar structures have also be found for Langmuir–Blodgett (LB)-films.[30] Those authors report that the formation of such structures is restricted to fatty acids with a large distance between the spin label and the carboxyl group because the flexibility of the alkyl chain has to be sufficient to enable the formation of these loops. This is in agreement with our findings: While 12- and 16-doxyl stearic acid films show the behavior described above, the 5-doxyl stearic acid film does not exhibit the equivalent behavior. Assuming a similar adsorption behavior of the spin label and the DTBN molecule studied earlier, one would expect that a large fraction of the molecules bound as loops are invisible for EPR spectroscopy. Furthermore the formation of loop structures should have important consequences

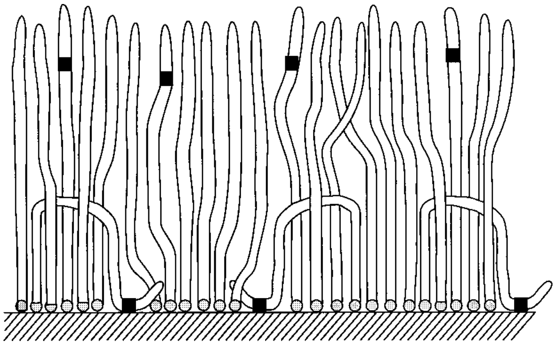

FIG. 12. Schematic representation of loop structures within a 16-doxyl stearic acid film. Squares represents the spin labels, hatched circles the head groups of the molecules.

for the structure of the film. First the adsorption of the spin labels blocks adsorption sites which are otherwise accessible to other fatty acid molecules. Due to size of the spin label and the steric demand of the alkyl chain this blockade may also concern several neighboring adsorption sites. Second, the intermolecular interactions between the chains are significantly disturbed caused by the formation of the loops. This effect influences the entire film. Whereas the inner part of the film, the part close to the substrate, is crowded by the presence of the additional molecules, the free space of the molecules in the outer part of the film is increased due to the lower number of molecules present. The dynamic behavior of the molecules should reflect this additional free space of the alkyl chains in the outer region of the film.

Comparing the lower spectrum of Fig. 10 with the equivalent spectrum of Fig. 3 no direct evidence for such a change in the dynamic behavior of the film is detected. At first glance the structures in the spectra connected with fast rotational motion of the molecules seem to be more distinct in the spectrum of the undisturbed films rather than in the disturbed one. However, on a closer examination of the spectra this is not confirmed. Whereas the simulation of the spectrum of the unperturbed film requires the assumption of static molecules (a fraction of 20%) in order to describe the line shape of the spectrum correctly, this is no longer necessary in the case of the disturbed spectrum. This is due to the fact that the A_z-component of the hyperfine tensor is shifted towards the isotropic value so that the anisotropy within the rotational constants decreases considerably from the unperturbed to the perturbed system. It is worthwhile to mention that an equivalent behavior, namely the shift of the A_z-component of the hyperfine tensor towards the isotropic value, was also found in the case of other perturbed systems as it has been discussed in the case of the temperature induced destruction of the 5-doxyl stearic acid and the dynamic behavior of the 12-doxyl stearic acid film. Looking at the component with the higher rotational constant necessary to simulate the spectrum of the perturbed film we find also a small increase in the relative intensity compared to the unperturbed film. The structure in the spectrum related to this component is less pronounced in the disturbed film because of the increase in linewidth of almost 2 G.

In order to explain the increase of the EPR intensity while increasing the temperature above room temperature we assume that the spin label desorbs from the surface with the carboxyl group remaining attached to the surface. Hence these molecules are detectable by EPR spectroscopy. Consequently the increase of the total number of spin labels will cause a decrease in the mean distance between the individual spin label as a three times larger amount of spin labels have to share the same space. Therefore the intermolecular spin–spin coupling and due to that the linewidth of the spectrum increases. The decrease of the Curie corrected intensity with decreasing temperature must be interpreted as a decrease of the number of EPR active spin labels. A decomposition of the spin labels as well as a desorption of the molecules would be unlikely while decreasing the temperature. Therefore we would suggest that a partial readsorption of the spin labels on the surface gives rise to this decrease of the Curie corrected intensity, indicating that the structure of the initially perturbed film does not seem to adopt the one observed for the unperturbed film at full coverage upon heating.

IV. SUMMARY AND CONCLUSIONS

In summary, we have discussed the temperature dependent EPR spectra of self-assembled stearic acid films containing different *n*-doxyl stearic acids as spin probes. The variation of the distance between the spin label and the surface changes the dynamic properties of the systems significantly. First, the onset for the rotational motion of the spin label is shifted drastically towards higher temperatures if the distance between the spin label and surface is reduced from 16- to 5-doxyl stearic acid. Second, the rotational excitation of the molecules decreases from 16- to 5-doxyl stearic acid for a given temperature. This qualitative statement was verified by computer simulations of the line shape. The simulations have shown that simple motional models assuming only two rotational constants are insufficient to account for the complex changes in the line shape of the spectra. Therefore the spectra have been simulated by a superposition of components with different rotational constants. Furthermore, the simulations have shown that the rotational motion is anisotropic in space. The comparison of the dynamic behavior of the 16- and 5-doxyl stearic acid films with the 12-doxyl stearic acid film show that the rotational behavior is strongly correlated to the order of the self-assembled film. This is corroborated by further experimental results where the order of the film was distorted thermally or through the preparation process itself.

The adsorption properties of 16- and 12-doxyl stearic acid differ considerably from those of the 5-doxyl stearic acid. The strong change of the adsorption behavior of the 16- and 12-doxyl stearic acid with temperature of the solution can be explained through the formation of loop structures were molecules form loops on the surface with both the carboxyl groups as well as the spin labels bound to it.

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[1] E. Meirovitch, A. Nayeem, and J. H. Freed, J. Phys. Chem. **88**, 3354 (1984).

[2] A. Lange *et al.*, Biochemistry **24**, 4383 (1985).

[3] A. A. Dubinski, G. G. Maresch, and H. W. Spiess, J. Chem. Phys. **100**, 2437 (1994).

[4] M. Romanelli *et al.*, J. Phys. Chem. **98**, 2125 (1994).

[5] H. Schlienz *et al.*, Phys. Rev. Lett. **74**, 761 (1995).

[6] U. J. Katter *et al.*, J. Phys. Chem. B **101**, 3776 (1997).

[7] H. W. Spiess, Ber. Bunsenges. Phys. Chem. **101**, 153 (1997).

[8] M. Farle, M. Zomack, and K. Baberschke, Surf. Sci. **160**, 205 (1985).

[9] T. Risse *et al.*, Langmuir **12**, 5512 (1996).

[10] D. L. Allara and R. G. Nuzzo, Langmuir **1**, 45 (1985).

[11] D. L. Allara and R. G. Nuzzo, Langmuir **1**, 52 (1985).

[12] D. March, in *Membrane Spectroscopy*, edited by E. Grell (Springer, Berlin, 1981), pp. 51–142.

[13] U. J. Katter, H. Schlienz, M. Beckendorf, and H.-J. Freund, Ber. Bunsenges. Phys. Chem. **97**, 340 (1993).

[14] R. M. Jaeger *et al.*, Surf. Sci. **259**, 235 (1991).

[15] M. Beckendorf, Ph.D. thesis, Ruhr–Universität Bochum, 1995.

[16] D. J. Schneider and J. H. Freed, in *Spin Labeling Theory and Applications*, Vol. 8 of *Biological Magnetic Resonance*, edited by L. J. Berliner and J. Reuben (Plenum, New York, 1989), Chap. 1, p. 1.

[17] D. J. Schneider and J. H. Freed, *Lasers, Molecules and Methods*, Vol. 73 of *Advances in Chemical Physics* (Wiley, New York, 1989), pp. 387–527.

[18] T. Risse *et al.*, J. Phys. Chem. (in press).

[19] U. J. Katter, Ph.D. thesis, Ruhr–Universität Bochum, 1995.

[20] J. F. W. Keana, Chem. Rev. **78**, 37 (1978).

[21] Z. Liang, P.-O. Westlund, G. Wikander, and G. Lindblom, Mol. Phys. **85**, 757 (1995).

[22] T. J. Schaafsma, Chem. Phys. Lett. **1**, 16 (1967).

[23] J. Skolnick and E. Helfand, J. Chem. Phys. **72**, 5489 (1980).

[24] E. Helfand and J. Skolnick, J. Chem. Phys. **77**, 5714 (1982).

[25] A. Ferrarini *et al.*, J. Chem. Phys. **91**, 5707 (1989).

[26] M. Ge and J. H. Freed, Biophys. J. **65**, 2106 (1993).

[27] E. Meirovitch *et al.*, J. Chem. Phys. **77**, 3915 (1982).

[28] A. Abragam, *The Principles of Nuclear Magnetism* (Oxford University Press, London, 1961).

[29] U. J. Katter *et al.*, J. Phys. Chem. B **101**, 552 (1997).

[30] F. Bonosi, G. Gabrielli, G. Martini, and M. F. Ottaviani, Langmuir **5**, 1037 (1989).

[31] B. J. Gaffney and H. M. McConnell, J. Magn. Reson. **16**, 1 (1974).