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# Analysis of Mobility of Protein Side Chains by Spin Label Technique

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**Abstract.** Five spin labeled derivatives of a neurotoxin from cobra venom were analyzed by the earlier suggested method. The procedure was adjusted to the complex motional behaviour of the label. Each protein derivative carried covalently bound spin label on different lysine residues. In two derivatives, at positions Lys44 and Lys46, the labels were strongly mobile, whereas for other three derivatives modified at Lys15, Lys25 and Lys26 the label was less mobile with respect to the protein molecule, which made possible determination of the rotational correlation time of the protein molecule (2.8 ± 0.3 ns). The rotational correlation time was in good agreement with the calculated value for the rigid sphere of the corresponding molecular weight. On the basis of the estimate of the anisotropic motion degree, it was found from the order parameter S that the label mobility increases in the following series of lysine residues: Lys26, Lys25, Lys15, Lys46, and Lys44. From the analysis of positions of outer wide peaks in ESR spectra obtained by varying temperature and viscosity of the medium, we determined the parameters for computer simulation. The theoretical and experimental spectra were found to be in good agreement.

**Key words:** Neurotoxin II from cobra venom – Spin-labeled lysines – Correlation time – Computer simulation of ESR spectra

## 1. Introduction

Spin label techniques have found wide application in molecular biology and biophysics (Likhtenstein 1976; Kurznetzov 1976; Berliner 1976). However, in order to obtain unambiguous information through the use of this method it is necessary to solve a number of problems in the interpretation of the ESR spectra of spin-labeled macromolecules. Complete elucidation of the experimental

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spectrum requires simulation until an adequate fitting is reached between the theoretical and experimental spectra parameters. It is difficult to decide which particular parameter should be programmed for computer operation and which program would be appropriate for such calculation basing on the spectrum line-shape only. Several authors (e.g., Berliner 1976; Polnaszec 1975; McCalley et al. 1972) put forward a number of simulation theories, however, examples of practical application are rather scarce. It may be due to the fact that simulation is connected with complicated mathematical software and computer operations. On the other hand, each specific case requires choosing an adequate label rotation model as well as finding the authentic parameters for its description. Interpretation of ESR spectra is often greatly complicated because several labels different in their mobility are simultaneously bound to one protein, which leads to inhomogeneity of the spectra.

In this work we analyzed the ESR spectra of a series of modified protein derivatives, each containing a single spin label positioned at a definite site. These derivatives were obtained by modification of neurotoxin II (mol. w. 6,880) of *Naja naja oxiana* cobra venom with N-hydroxysuccinimide ester of the pyperidine series spin label. They are labeled at the following lysine residues: Lys15, Lys25, Lys26, Lys44 and Lys46 (Tsetlin et al. 1979a).

ESR analysis was based on the resulting set of experimental spectra at varied temperature and viscous media. Variation of these conditions allowed to obtain distinct OWPs, their occurrence being the criteria of the immobilized spectra. It was earlier shown by (Dudich et al. 1977; Timofeev et al. 1980; Wasserman et al. 1981) that analysis of the OWP positions is the masterkey to the interpretation of ESR spectra. The temperature/viscosity dependence of distances between OWPs permitted the distinction of the contributions in an ESR spectrum to be made for Brownian macromolecular rotation and for rapid anisotropic motion of labels about the protein moiety. This discrimination, in its turn, enables one to precisely determine the correlation time of the protein molecules and the degree of anisotropic rotation of the spin label, which makes it possible to characterize the state of the lysine residue side chain binding the label. The correct choice of the spin motion model is proved by comparison of the theoretically calculated spectra to the experimental ones.

## 2. Materials and Methods

### *2.1 Proteins and ESR Technique*

The methods for preparing the spin labeled derivatives, their separation and identification of the label positions were described earlier (Tsetlin et al. 1979). The ESR spectra were measured on a Varian E-104A spectrometer at microwave power of 5 mW and modulation amplitude 1 G and scan range 200 G. The OWPs were recorded separately at a 2-G modulation amplitude and scan range of 100 G. Lyophilized spin labeled proteins were dissolved in a *Tris*-HCl buffer, pH 7.0 concentration $(1-2) \cdot 10^{-4}$ M. The viscosity was varied by adding crystalline sucrose (Merck). The sucrose concentration in a sample was

measured with a refractometer. The results were processed as described earlier (Dudich et al. 1977; Timofeev et al. 1980).

### *2.2 Method for Determining the Magnetic Parameters for Computer Simulation of ESR Spectra*

Earlier it was shown (Dudich et al. 1977) that in the temperature range from 0° to 40° C and viscosity interval of sucrose concentrations from 0 to 40% the following condition is fulfilled for the spin label bound to a macromolecule:

$$\tau_L \ll \tau,\ \tau_L < 10^{-9}\ \text{s}. \tag{1}$$

As a consequence of this condition, the total OWP shift in the ESR spectra is represented by two contributions with isothermic change in the viscous medium

$$\Delta = (2A_z - 2A') = (2A_z - 2\bar{A}) + (2\bar{A} - 2A') = \Delta_s + \Delta_\tau. \tag{2}$$

The first $\Delta_s$ is the OWP shift due to the rapid anisotropic rotation of the radical in respect of the protein molecule. The existence of the rapid anisotropic rotation results in partial averaging of tensors $\hat{A}$ and $\hat{g}$. Such an averaging may be quantitatively described via the order parameter $S$ in the following way:

$$S = \frac{\bar{A} - \bar{A}_\perp}{A_z - A_\perp} = \frac{\bar{A} - a_0}{A_z - a_0} \tag{3}$$

or in terms of the effective rotation cone:

$$S = {}^1\!/_2\,(\cos^2\alpha + \cos\alpha), \tag{3'}$$

where

$$a_0 = {}^1\!/_3\,(A_z + 2\,A_\perp) = {}^1\!/_3\,(\bar{A} + 2\,\bar{A}_\perp) \tag{4}$$

and $\alpha$ is half amplitude of the effective cone motion. The second contribution is due to slow rotation of the protein and equal to

$$\Delta_\tau = C \cdot \tau^{-\beta}, \tag{5}$$

where $\tau = \dfrac{V\eta}{\varkappa T}$ (according to the Stokes-Einstein law); $C$ is constant; $\beta$ = the exponent weakly depending on $S$; $V$ = the macromolecule volume.

Thus, using Eqs. (2–5), the total OWP shift assumes the form

$$\Delta = 2(1 - S)(A_z - a_0) + C \cdot \tau^{-\beta}. \tag{6}$$

From Eq. (6) it is seen that the total $\Delta$ OWP shift under condition (1) and constants $A_Z$ and $a_0$ for the radical involved depends on the parameter $S$ and on the rotational correlation time. Eq. (2) is also valid for IWPs, taking into account that $2\bar{A}_\perp < 2A'_\perp$ and Eq. (5) has its own $\beta_\perp$. $A_Z$ is determined experimentally as the distance between OWPs in the spin labeled samples at 77 K, whereas $a_0$ is determined as the distance between the adjacent ESR lines of the triplet spectrum from the free radical in solution. When plotting the dependence of $2A'$ versus the $T$ and $\eta$ at constant $T$ one can seen that with sucrose up to 40% (~ 6 sps) the curve will be linear since condition (1) is still observed. Therefore, $2A'$ tends to $2\bar{A}$, when $\eta \to \infty$. However if the sucrose content exceeds 40%, $\tau_L$ becomes comparable to $10^{-9}$ s, and $2A'$ will then tend to $2A_z$ with $\eta \to \infty$. By extrapolating the linear plot to the intersection point on the ordinate, we obtain $2\bar{A}$ which enables us to calculate the parameter $S$ using formula (3). Similarly, we can plot $2A_\perp$ versus $T$ and $\eta$ of solution, and thus obtain $2\bar{A}_\perp$.According to formula (4) $a_0$ is calculated which allows one to estimate the polarity of the spin label environment. By inserting experimental parameters $A_z$, $a_0$ and $S$ and the $\Delta g$ value of the $g$-factor anisotropy (i.e., the effective axial symmetrical components of tensors $\hat{A}$ and $\hat{g}$) as well as the residual width WL, into McConnel or Freed program in order to simulate ESR spectra (Berliner 1976), one obtains theoretical spectra similar to experimental, which reflect their peculiarities both quantitatively and qualitatively.

## 3. Results

Experimental ESR spectra of the spin labeled protein derivatives are illustrated in Figs. 1–5. The Figs. 1–3 (A) present the ESR spectra at different temperatures, and (B) – the ESR spectra obtained at isothermal change of viscosity. Figure 6 exhibits the OWP and IWP separations (where they can be determined) depending on $(T/\eta)^{0.74}$ and on $(T/\eta)^{0.45}$, respectively, at 1° C. The ESR parameters obtained from Fig. 6 and from the experimental ESR spectra, as well as the calculated correlation times, $\tau$, are given in Table 1.

The circled numbers in Figs. 1–3 correspond to those in Fig. 6. Thus, separations $2A'$ and $2A'_\perp$ for OWP and IWP are correlated with their spectra. For all spectra, the value $\tau$ found from the shift $\Delta_\tau = 2\bar{A} - 2A'$ in the nomogram (Dudich et al. 1977; Timofeev et al. 1980; Wasserman et al. 1981) is given for viscosity media (% sucrose) and parameter order $S$. Figure 7 represents ESR spectra simulated from experimental parameters (see Table 1 for labeled Lys26). Parameters $S$ were calculated from formula (3), assuming $2A_Z = 74.5$ G and $a_0 = 17.1$ G; values $2\bar{A}$ and $2\bar{A}_\perp$ were obtained by extrapolating the lines in Fig. 6A and 6B, accordingly. The isotropic constant $a_0$ was calculated from formula (4). From the nomogram (Timofeev et al. 1980) the value $\tau$ was measured for each point on the straight lines with $\beta = 0.74$ and $\beta = 0.45$ (as for the first three samples $S$ approaches 1) for the corresponding $2\bar{A} - 2A'$ difference. The same slope in Fig. 6 affords the value $\tau$ for Lys26, Lys25, and Lys15 derivatives with $\tau$ reduced to normal condition $2.8 \pm 0.3$ ns.

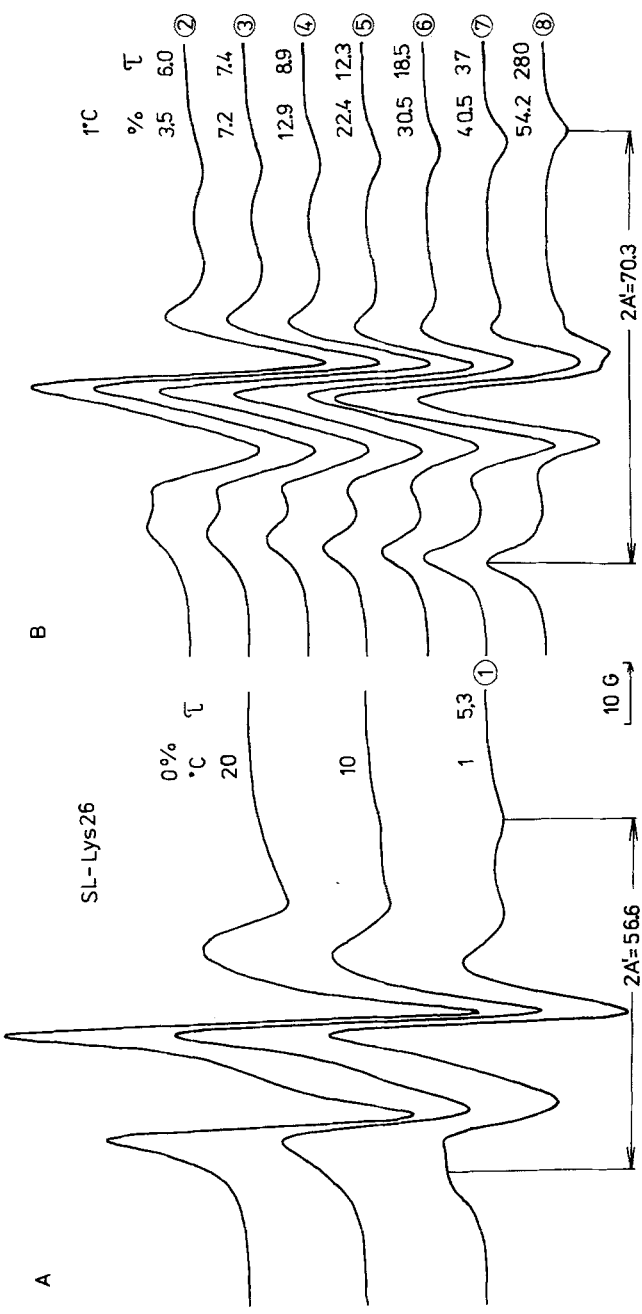



**Fig. 1 A and B.** ESR spectra of SL-Lys26-NTII. **A** for three temperatures in the absence of sucrose, **B** for different sucrose concentrations and at constant temperature. The Figure exhibits the temperature and % sucrose at which the spectrum was recorded and the correlation time of the protein molecule ($\tau$ in ns) experimentally measured

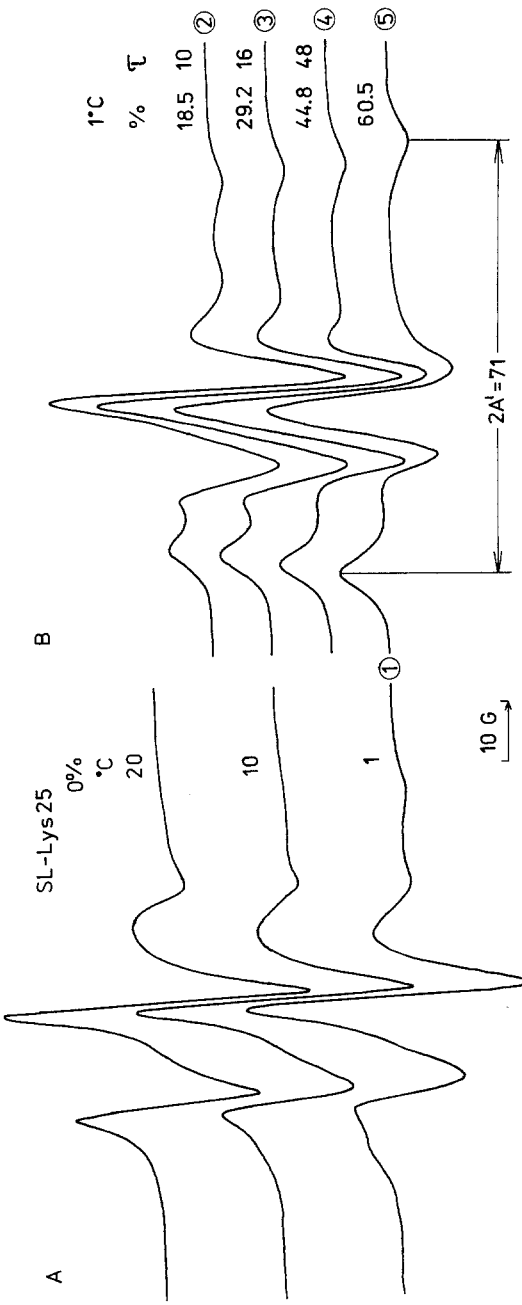



**Fig. 2.** ESR spectra of SL-Lys25-NTII (see Fig. 1 inscription)

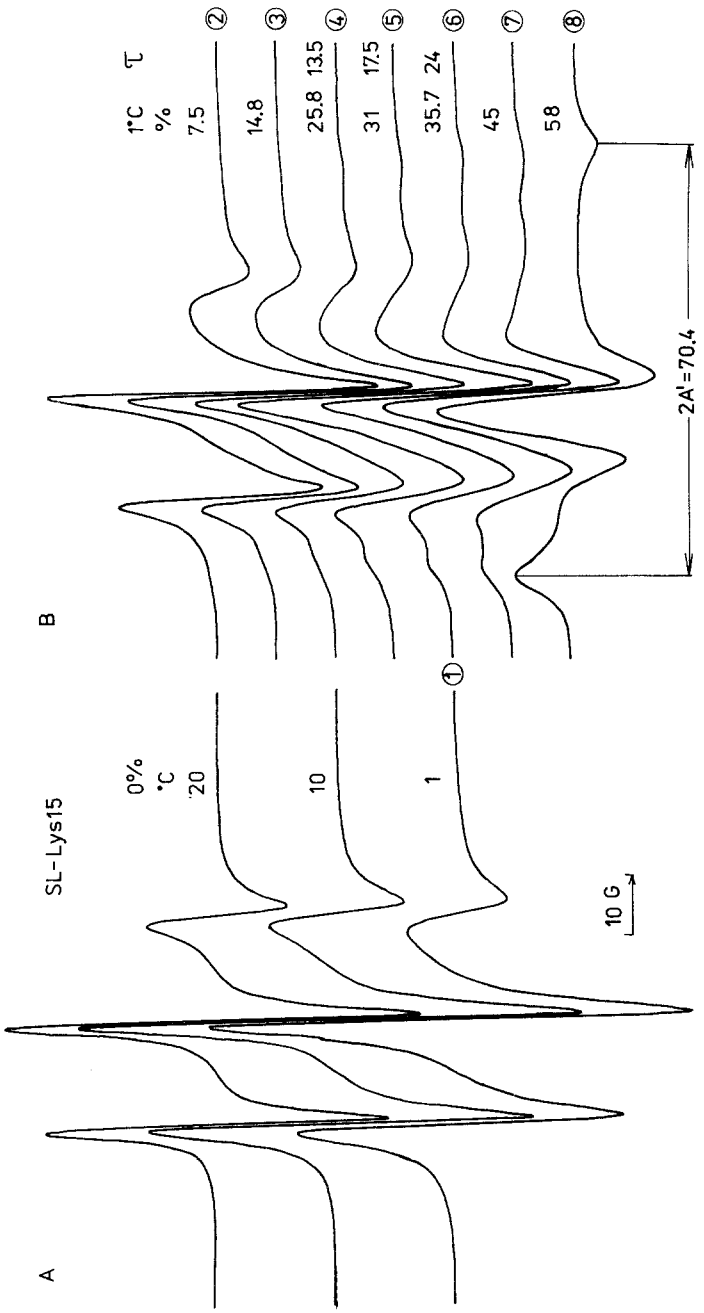



**Fig. 3.** ESR spectra of SL-Lys15-NTII (see Fig. 1 inscription)

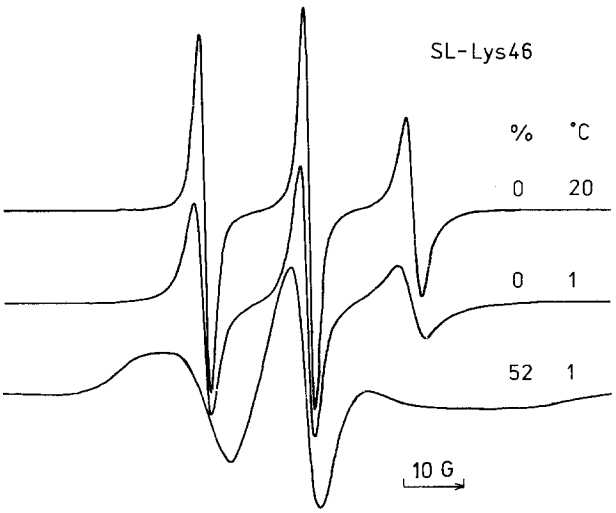



**Fig. 4.** ESR spectra of SL-Lys46-NTII as dependent on temperature (° C) and viscous medium (% sucrose)

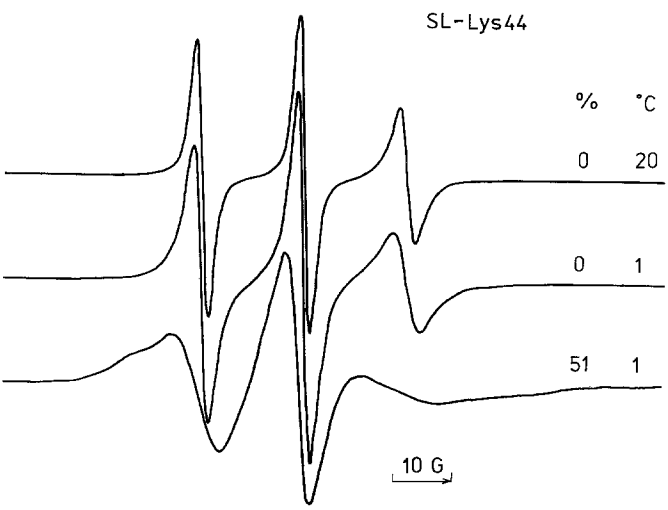
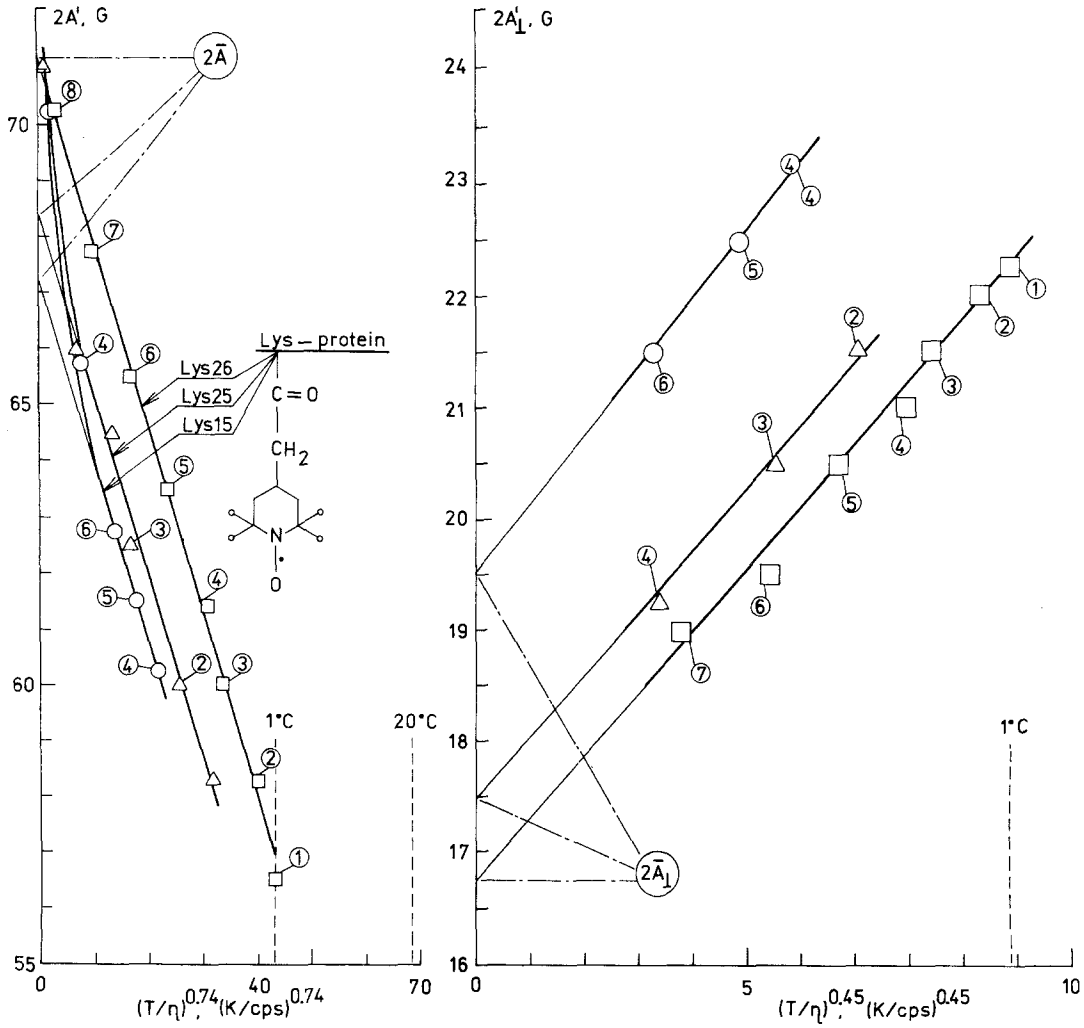



**Fig. 5.** ESR spectra of SL-Lys44-NTII as dependent on temperature (° C) and viscous medium (% sucrose)




**Figs. 6A and B.** Dependence of OWP **(A)** and IWP **(B)** distances in the ESR spectra of the protein for the following spin label positions: Lys26 (□), Lys25 (△) and Lys15 (○) on the viscous medium at 1° C

**Table 1.** ESR spectral parameters and rotational correlation times of spin labeled protein derivatives

| Protein modification at | ° C | 2 $A$ [G] | $S$ | $\alpha$ | 2 $A_t$ [G] | $\alpha_0$ [G] | $\tau$ [ns] | $\tau$ 20° C [ns] |
|---|---|---|---|---|---|---|---|---|
| Lys 26 | 20 | 66.3 | 0.79 | 32 | – | – | 2.8 | 2.8 |
| | 10 | 69.5 | 0.88 | 22 | 17.8 | 17.5 | 3.8 | 2.8 |
| | 1 | 71.0 | 0.92 | 17 | 16.9 | 17.4 | 5.3[a] | 2.8 |
| Lys 25 | 10 | 66.0 | 0.79 | 32 | 19.5 | 17.5 | 3.8 | 2.8 |
| | 1 | 68.5 | 0.84 | 27 | 17.5 | 17.3 | 5.3 | 2.8 |
| Lys 15 | 1 | 67.0 | 0.81 | 30 | 19.5 | 17.6 | 5.3 | 2.8 |

[a] The value of $\tau$ was obtained from the shift $2\bar{A}-2A'$ at 0% sucrose (see Fig. 6A). The other values of $\tau$ were obtained from the shift at corresponding values of viscosity (see % sucrose from Fig. 6A) and reduced to a normal conditions at 20° C and 0% sucrose

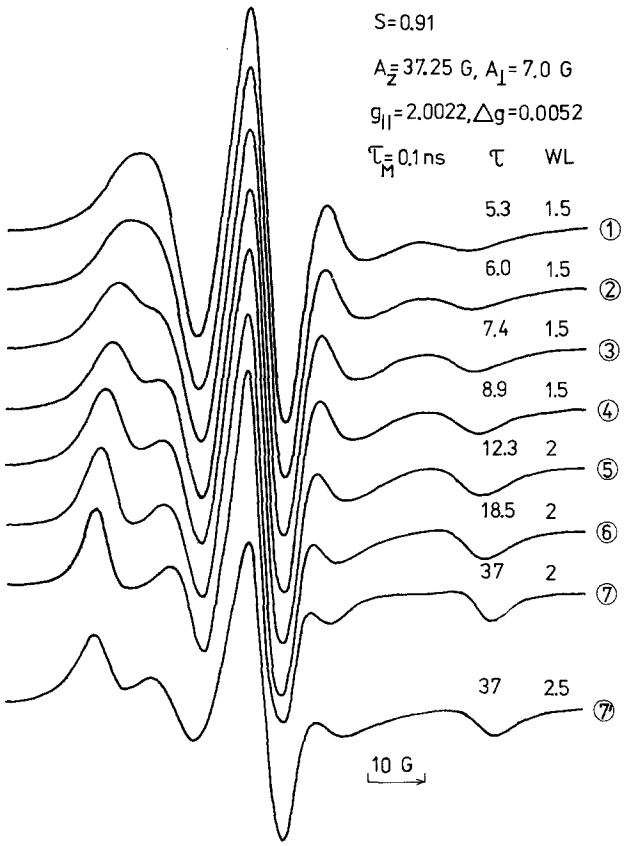



**Fig. 7.** Theoretical ESR spectra. ①–⑦ represent the spectra calculated from the Freed program (in Berliner 1976). ⑦ is the spectrum calculated by McConnell method (McCalley et al. 1972)

## 4. Discussion

### *4.1 ESR Spectra Degenerated in τ and S for the Spin Labeled Macromolecule*

As seen from Figs. 1A, 2A, 3A, 4, and 5 the spectra of all five spin labeled derivatives under normal condition (20° C and 0% sucrose) are characteristic for a mobile label. It is not surprising because for a 7,000 dalton protein molecule the rotational correlation time should be several ns, and the ESR spectrum of the spin label which is rigidly bound to such a molecule ($\tau_L = \tau$) must have mobile spectral line shape – a triplet with different peak amplitudes. Moreover, spin labels introduced at lysine residues into the protein molecule of a larger mol.w. (100,000) usually produce a rapid tumbling ESR spectrum (Griffith and McConnell 1966) due to the fact that ε-amino groups of lysine residues are, as a rule, rather mobile with respect to the protein moiety.

Comparison of line shapes in ESR spectra taken at 20° C shows that all of them have a common mobile spectral form, however, the spectra of the Lys25

and Lys26 derivatives are less mobile than the rest. The differences in the spectra were considered earlier within the isotropic rotation model of the label about protein (Tsetlin et al. 1979a). In addition, it was also shown that the spin label had more limitations in its mobility for Lys26 and Lys25.

The differences in the character of motion for the spin label derivatives under study become more pronounced in the ESR spectra if the temperature is lowered to 1° C in which case the spectra of samples labeled at positions Lys26 and Lys25 (Figs. 1A and 2A) are strongly modified. However, the rest of the samples maintain their original line shape. For these two derivatives, appearance of OWP is clearly seen, but these OWPs are not completely resolved due to their overlapping with sharp peaks. If it is remembered that all protein derivatives are characterized by approximately the same $\tau$, a conclusion can be made on the basis of differences in the spectra about the unlikeness of label immobilization with respect to the protein moiety. To make a qualitative estimate of the contribution to the ESR spectra of the slow tumbling protein molecule and a more rapid label motion, the technique elaborated in our laboratory (see "Materials and Methods") was used. For interpretation of the spectrum, it is sufficient to follow the OWP shifts when the viscosity of solution is isothermally enhanced with addition of sucrose as suggested by the luminescence polarization method (Whal and Weber 1967). For this purpose the temperature should be decreased and the viscosity increased till OWPs showed up. Only then the viscosity of solution must be raised gradually at a constant temperature. It should be noted that if the sucrose concentration is below 40%, OWPs will show up for another derivative spin labeled at Lys15 (Fig. 3B), but for Lys46 and Lys44 practically no OWPs were observed. However, the line shape of the spectra of the last two derivatives indicates that the Lys46 residue is more immobilized than the Lys44 residue. If the sucrose concentration is above 40%, condition (1) is violated, i.e., the label begins to rotate less rapidly in a limited motion cone with respect to the protein moiety, and in this case we observe a deviation from the linear dependence in the plots illustrated in Fig. 6.

The half amplitude of the effective cone rapid motion [formula (3′)] can be found for any attached label by determining parameter $S$.

As a result, the line shape of initial spectra for all neurotoxin derivatives were found to be degenerated in two parameters $\tau$ and $S$. In order to get rid of this degeneracy, a dependence $2A'$ versus $T$ and $\eta$ should be plotted by varying isothermically $\eta$ so that this separation is yet determined. Figures 1, 2 and 3B show a set of immobilized ESR spectra from which the separation $2A'$ was determined and the temperature/viscosity dependences (Fig. 6) plotted. The corollaries of the proposed model are quite clear. Firstly the tighter attachment of the label to protein, the less is needed the medium viscosity for obtaining an immobilized spectrum. For Lys26, the straight line begins at 0% sucrose in Fig. 6, for Lys25 at about 10%, and for Lys15 only at 26%. Parameter $S$ was determined from extrapolated values $2\bar{A}$ (see Table 1). Theoretically, for Lys46 and Lys44 (Dudich et al. 1977) parameter $S$ must be smaller than 0.5 (or $\alpha = 53°$), i.e., the value at which OWP should not appear, which is proved experimentally.

Secondly, the tighter attachment of the label to protein, i.e., the weaker temperature dependence for $2\bar{A}$, the less is the temperature activation of the label, and vice versa. From Table 1 it can be seen that $2\bar{A}$ is found at 1°, 10°, and 20° C for Lys26, for Lys25 at 1° and 10° C, for Lys15 only at 1° C. For Lys46 and Lys44 it cannot be determined at all. Hence, the degree of immobilization of side chains also manifests itself in temperature activation of spin labels covalently bound to them. In other words, the smaller parameter $S$, the lower is the % sucrose range for the OWP in ESR spectra of spin labeled samples and the higher temperature activation of side chains, by a full analogy to the luminescence polarization method (Whal and Weber 1967).

It was possible thus to determine the correlation time (Fig. 6) using only three spin labeled protein derivatives (at Lys26, Lys25, Lys15). The reliability of the approach is proved by the fact that the same value was obtained in three cases. The resulting $\tau$ ($2.8 \pm 0.2$ ns) is in good agreement with the rotational relaxation time (9 ns) as determined from the Stokes-Einstein law with account of the partial specific volume of constituent amino acids and water transported by the protein molecule during the Brownian rotational diffusion motion (Surin et al. 1981).

### *4.2 Comparison of Spin Label and Fluorescence Polarization Methods*

At this point it is reasonable to discuss the similarity of the fluorescence polarization and the spin label method. Following Weber (Whal and Weber 1967) the value

$$\bar{Y} = \left(\frac{1}{p_0} - \frac{1}{3}\right) \Big/ \left(\frac{1}{p_0'} - \frac{1}{3}\right)$$

characterizes the average amplitude of rapid reorientation of the dye relative to the protein where $P_0 = (I_\parallel - I_\perp) / (I_\parallel + I_\perp)$ and $P_0' = (\bar{I}_\parallel - \bar{I}_\perp) / (\bar{I}_\parallel + \bar{I}_\perp)$; $P_0$ is the rigid-limit degree of fluorescence polarization for the given medium, $P_0'$ – the degree of polarization resulting from the intercept of the experimental straight dependence $P = (I_\parallel' - I_\perp') / (I_\parallel' + I_\perp')$ on $T/\eta$ at $\eta \to \infty$, $I_\parallel$ and $I_\perp$-intensities of emitted fluorescence light in the parallel and perpendicular direction for over-all immobilized dyes, $\bar{I}_\parallel$ and $\bar{I}_\perp$ represent the same constitutents of $I_\parallel$ and $I_\perp$ but only partially averaged because of rapid reorientation of dye at limited angles; $I_\parallel'$ and $I_\perp'$-components for the given $T$ and $\eta$. The difference between the extrapolated polarization $P_0'$ and fundamental polarization $P_0$ is explained (Weber and Whal 1967) by the side chain motion. Indeed, one can easily see that value $\bar{Y}$ can be re-written as follows: $(\bar{I}_\parallel - \bar{I}) / (I_\parallel - I_\perp)$ [cf. with $S$ in formula (3)], while Eq. (5) is similar to the Perrin-Weber equation for finding the rotational relaxation time $\varrho = 3\tau$ (Whal and Weber 1967):

$$\left(\frac{1}{p} - \frac{1}{3}\right) = \left(\frac{1}{p_0'} - \frac{1}{3}\right)\left(1 + \frac{3\tau_0}{\varrho}\right) \quad \text{or} \quad I_\parallel' - I_\perp' = (\bar{I}_\parallel - \bar{I}_\perp) \Big/ \left(1 + \frac{3\tau_0}{\varrho}\right),$$

where $\tau_0$ is the lifetime of the excited state of the fluorescence. Consequently, the study of behaviour of OWPs by spin label technique is the same as for polarized emission by steady state fluorescence polarization method.

The values of half amplitudes of the effective cone motion obtained at 1° C are equal to 17,25 and 30° C for spin labels bound to ε-amino acid lysine residues 26, 25, and 15. Thus, the mobility of labels is increased in the following order: Lys26, Lys25, Lys15, Lys46, and Lys44. The same order was reported earlier when analyzing ESR spectra recorded at 20° C within the model for isotropic rotation (Tsetlin et al. 1979a). The data obtained show that the labels at positions Lys26 and Lys25 experience considerably stronger steric hindrances than those at other positions. We can assume this conclusion to be valid not only for labels, but also for the side groups to which they are bound. Noteworthy, a limited mobility for ε-amino group of the Lys26 residue in a homologous toxin was demonstrated by NMR spectroscopy (Inagaki et al. 1980).

The limitations of mobility of labels at positons Lys26, Lys25, and Lys15 could indicate that they are located inside the protein globule, but this is not the case. The $a_0$ value (see Table 1) derived from the ESR spectra using formula (4) (see "Materials and Methods") evidences that the labels, in fact, exist in polar aqueous environment – probably, on the protein surface. For Lys46 and Lys44 derivatives it is difficult to measure the $a_0$, due to the absence of OWPs in the spectra, because of strong mobility of the spin labels at these residues; this, in its turn, confirms their exposure. Obviously, the limitation of mobility of bound label results from the spatial proximity to side chains of the adjacent amino acid residues located on the surface. In this connection, it is appropriate to mention two facts. Firstly, the X-ray studies on the homologous neurotoxin, erabutoxin b showed that the molecule is rather flat in the shape and that it is difficult to state the presence of a real hydrophobic core (Kimball et al. 1979). Secondly, for neurotoxin II fluorescence and NMR analyses demonstrated that the Lys44 side chain had no groups in its immediate vicinity whereas the spin labels at positions Lys25 and Lys26 experienced the influence of many closely-ranged groups; in particular, the indole rings of Trp27 and Trp28 residues are spaced at a distance of no more than 4–6 Å from them (Surin et al. 1981; Tsetlin et al. 1979b).

### *4.3 Spin-Label as a Molecular Ruler*

In the light of the results obtained it is of interest to analyze the merits and limitations of spin labels used as "molecular ruler" (see, for example, Hsia and Piette 1969; Willan et al. 1977; Hull et al. 1975; Defaye et al. 1980; Cornell and Kaplan 1978). The measurement of correlation times is normally made by comparing the ESR spectra of spin labeled proteins in which spin labels are bound to one and the same protein group by means of alkylating arms of different lengths, to the theoretical spectra for the isotropic rotation model of radicals. The depth for alkylated group is found from the $\tau$ dependence versus the length of the arm. For all the reported proteins, the determined depth was

10–14 Å. The persistency of this value seems strange and can be explained by equivocal interpretation of ESR data. It should be taken into account that $\tau$ for protein is not changed upon varying the label arm. Therefore, the changes observed in the spectra, when the arm is varied, seem to result from rapid re-orientation of the label, i.e., from the changes in parameter $S$. For neurotoxin, it is likely that the group lying on the surface of the protein was modified, whereas the changes of the spectra upon varying the length of the arm depend on the label contact with immediate protein environment. In our opinion, the method of molecular rulers provides adequate information about the depth of some groups in membranes. For soluble proteins of relatively small molecular weight this method affords only qualitative information about the character of protein environment of the groups involved. Therefore, the method of molecular rulers is not always useful for measuring the depths from protein surface.

### *4.4 Simulation of ESR Spectra of the Spin Labeled Macromolecule*

In this study we did not set ourselves the aim to computer simulate the ESR spectra for all the neurotoxin derivatives. In Fig. 7, seven spectra are given only for the neurotoxin derivative spin labeled at position Lys26. They were simulated according to Freed program (Berliner 1976) by inserting the axial-symmetrical parameters of tensors $\hat{A}$ and $\hat{g}$ averaged on the account of rapid limited reorientation of the label. The circled numbers in Fig. 7 near spectra correspond to those in Figs. 1A and 1B. One can see quantitative and qualitative consistency between the theoretical and experimental spectra, except that there is a low-field doubled peak in the experimental spectra ① and ②, whereas in the theoretical ones it is absent. This is apparently due to the fact that the axis of rapid label rotation used in Freed program coincides with that for the main magnetic tensors. It is possible to achieve a better fit (for small $\tau$ values) if the Polnaszek-Freed program (Polnaszek 1975) is taken. Spectrum ⑦ is simulated via McConnel programm. As is seen, the spectrum has the same basic features as spectrum ⑦ however it simulates the experimental spectrum somewhat worse. It was expected as the simulation spectra from McConnell programm coincide worse with the experimental ones in the central part of the spectrum for $\tau$ smaller than 10 ns. It should be noted that by varying $\Delta g$ and WL it is possible to achieve a better theoretical and experimental fit, leaving other parameters invariable. In the same manner, it is possible to simulate the spectra for other derivatives (for parameters see Table 1).

Thus, the results obtained in this study illustrate the vitality of the method. It is being further developed for analysis of ESR spectra. The results obtained for neurotoxins are in good agreement with information provided by other physico-chemical techniques.

*Nomenclature*

$\tau$ – rotational correlation time of the protein molecule;
$\tau_L$ – rotational correlation time of the spin label;
$2A_Z$, $2A_\perp$ – the rigid limit distance between OWP and IWP, respectively, for ESR spectra of spin labeled proteins.
$2\bar{A}$, $2\bar{A}_\perp$ – the averaged limit distance between the OWP and IWP correspondingly mobile spin label to respect of protein moiety with; $\tau = \infty$
$2A'$, $2A'_\perp$ – distance between OWP and IWP in the ESR spectra of spin labeled proteins for any $T$ and $\eta$ media.

*Abbreviations*

SL – spin label;
NT – neurotoxin II from cobra venom;
NT-SL-Lys44 – neurotoxin spin labeled at Lys44 residue;
OWP – outer wide peaks in the immobilized ESR spectra;
IWP – inner wide peaks in the immobilized ESR spectra;
WL – the residual linewidth.

## References

Berliner LJ (1976) Spin labelling. Theory and Application. Academic Press, New York
Cornell CN, Kaplan LJ (1978) Spin label studies of sulfhydryl environment in bovine plasma albumin 1. The NF transition and acid expansion. 2. The neutral transition and the A isomer. Biochemistry 9: 1750–1758
Defaye G, Basset, Monnier N, Chambaz EM (1980) ESR study of human transcortin thiol groups and site topography. Biochim Biophys Acta 623: 280–294
Dudich IV, Timofeev VP, Volkenstein MV, Misharin AYu (1977) Macromolecule rotational correlation time measurement by ESR method for covalently bound spin-label. Mol Biol (USSR) 9: 531–538
Griffith OH, McConnell HM (1966) A nitroxide-maleimide spin label. Proc Natl Acad Sci USA 55: 8–11
Hsia JC, Piette LH (1969) Spin-labeling as a method in studying antibody active site. Arch Biochem Biophys 129: 296–307
Hull HH, Chang R, Kaplan LJ (1975) On the location of the sulfhydryl group in bovine plasma albumin. Biochim Biophys Acta 400: 132–136
Inagaki F, Tamiya N, Miyasawa T (1980) Molecular conformation and function of erabutoxins as studied by nuclear magnetic resonance. Eur J Biochem 109: 129–138
Kimball MR, Sato A, Richardson JS, Rosen LS, Low BW (1979) Molecular conformation of erabutoxin b: atomic coordinates at 2.5 Å resolution. Biochem Biophys Res Commun 88: 950–959
Kuznetzov AN (1976) A methods of spin probe. Nauka Moscow
Likhtenstein GI (1976) Methods in Molecular Biology. Wiley-Interscience, New York
McCalley RC, Shimshik EI, McConnell HM (1972) The effect of slow rotational motion on paramagnetic spectra. Chem Phys Lett 13: 115–119
Polnaszek CF (1975) Ph. D. Thesis, Cornell University
Surin AM, Utkin YuN, Pluzhnikov KA, Efremov ES, Tsetlin VI (1981) Fluorescence studies of neurotoxin II from the venom of cobra *Naja naja oxiana* and its derivatives: conformation of the fragment $-Lys^{25}-Lys^{26}-TRP^{27}-Trp^{28}$. Biorgan Khim (USSR) 7: 342–356
Timofeev VP, Dudich IV, Volkenstein MV (1980) Comparative study of dynamic structure of pig and chicken aspartate aminotransferases by measuring the rotational correlation time. Biophys Struct Mech 7: 41–49

Tsetlin VI, Karlsson E, Arseniev AS, Utkin YuN, Surin AM, Pashkov VS, Pluzhnikov KA, Ivanov VT, Bystrov VF, Ovchinnikov YuA (1979a) EPR and Fluorescence study of interaction of *Naja naja oxiana* neurotoxin II and its derivatives with acetylcholine receptor protein from *Torpedo marmorata.* FEBS Lett 106: 47–52

Tsetlin VI, Arseniev AS, Utkin YuN, Gurevich AZ, Senyavina LB, Bystrov VF, Ivanov VT, Ovchinnikov YuA (1979b) Conformational studies of neurotoxin II from *Naja naja oxiana:* selective N-acylation, circular dicroism and nuclear-magnetic-resonance study of acylation products. Eur J Biochem 94: 337–346

Wasserman AM, Aleksandrova TA, Dudich IV, Timofeev VP (1981) An investigation of the segmental flexibility of spin labeled synthetic macromolecules in solution. Eur Polymer J 17: 347–352

Whal P, Weber G (1967) Fluorescence depolarization of rabbit gamma globulin Conjungates. J Mol Biol 30: 371–382

Willian KJ, Marsh D, Sunderland CA, Sutton BJ, Wain-Hobson S, Dwek RA, Givol D (1977) Comparison of the dimensions of combining sites of the dinitrophenyl-binding immunoglobulin A myeloma proteins MOPC 315, MOPC 460 and XRPC 25 by spin-label mapping. Biochem J 165: 199–206

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