

Aggregation of Trichogin Analogs in Weakly Polar Solvents: PELDOR and ESR Studies

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Received May 8, 2000; revised June 6, 2000

Abstract. The technique of pulsed electron-electron double-resonance is used to study the self-aggregation of spin labeled trichogin GA IV analogs in weakly polar solvents. The dipole-dipole spin-spin relaxation of spin labels has been experimentally studied in glassy solutions of spin-labeled peptides frozen to 77 K in the mixtures of chloroform-toluene, chloroform-decalin, tetrachloromethane-toluene, dichloroethane-toluene depending on the label position in peptide and the structure of terminal groups. It is shown that the studied trichogin analogs in weakly polar solvents form aggregates whose structure depends on solvent properties and peptide structure. It is also demonstrated that the distances between spin labels which can be measured in aggregates amount to the values of 2.3, 2.6 and 3.3 nm. The lower estimate is given for the average number of peptide molecules in aggregates to within 3.1–4.3 depending on peptide structure and solvent composition.

1 Introduction

Recently, the methods of pulsed electron spin resonance (ESR) spectroscopy have been used to analyze the structure of spin-labeled peptides in frozen glassy solutions by extracting information about the distances between spin labels on the basis of measurements of the parameters of dipole-dipole couplings between them. Milov et al. [1, 2] combined the method of pulsed electron-electron double-resonance in electron spin echo (PELDOR) with the continuous wave (cw) ESR to study the spin-labeled analogs of trichogin GA IV. In these studies, the PELDOR technique was used to investigate the dipole-dipole interactions between spin labels for mono- and double-labeled peptides in frozen glassy solvents such as alcohols and the mixture of chloroform with dimethylsulfoxide. These works demonstrated the potentialities of PELDOR application for structural studies of spin-labeled peptides.

This technique was used to reveal the aggregation of mono-labeled trichogin analogs in weakly polar solvents [3]. With peptides III and V, whose structure is shown in Table 1 and Fig. 1, we have experimentally studied the dipole-di-

Table 1. Sequences of amino acid residues for trichogin GA IV and its spin labeled analogs I–VII. The structures of Lol, TOAC, Aib, Fmoc and TEMPON are shown in Fig. 1.

Trichogin GA IV		<i>n</i> -Oct-Aib-Gly-Leu-Aib-Gly-Gly-Leu-Aib-Gly-Ile-Lol
I	FTOAC 1.8	Fmoc-TOAC-Gly-Leu-Aib-Gly-Gly-Leu-TOAC-Gly-Ile-Leu-OMe
II	FTOAC 1	Fmoc-TOAC-Gly-Leu-Aib-Gly-Gly-Leu-Aib-Gly-Ile-Leu-OMe
III	FTOAC 4	Fmoc-Aib-Gly-Leu-TOAC-Gly-Gly-Leu-Aib-Gly-Ile-Leu-OMe
IV	FTOAC 8	Fmoc-Aib-Gly-Leu-Aib-Gly-Gly-Leu-TOAC-Gly-Ile-Leu-OMe
V	TOAC 1	<i>n</i> -Oct-TOAC-Gly-Leu-Aib-Gly-Gly-Leu-Aib-Gly-Ile-Leu-OMe
VI	TOAC 4	<i>n</i> -Oct-Aib-Gly-Leu-TOAC-Gly-Gly-Leu-Aib-Gly-Ile-Leu-OMe
VII	TOAC 8	<i>n</i> -Oct-Aib-Gly-Leu-Aib-Gly-Gly-Leu-TOAC-Gly-Ile-Leu-OMe

pole spin-spin relaxation of spin labels of these peptides in a chloroform-toluene mixture frozen down to 77 K. A fast decay of the PELDOR signal at short times with subsequent signal oscillations allowed conclusions regarding peptide aggregate formation, estimation of the mean number of peptide molecules in peptide cluster and determination of some characteristic distances between spin labels in aggregates. The cw ESR data obtained previously [3] testify to a possible existence of aggregates in the same solutions at room temperature. It has been established that the addition of ethanol as a polar additive to solution causes aggregate destruction.

The PELDOR technique, given in more detail previously [4], is a modification of the electron spin echo (ESE) method. The PELDOR signal is a usual ESE signal measured by switching on additional pumping microwave (mw) pulse to change the dipole-dipole interaction of spins. Two mw pulses induce the ESE signal at frequency ω_A in a spin system. Between these two pulses the pumping pulse at frequency ω_B is applied at time T after the first pulse. The spins are labeled as spins A (at ω_A) and spins B excited by the pumping pulse at frequency ω_B . The pumping pulse induces transitions between the Zeeman levels of spins B and thus changes local magnetic fields at spins A. This, in turn, results in the additional dephasing of spins A and, hence, a decrease in the ESE amplitude. This decrease depends on the value of dipole-dipole spin coupling and the time position and intensity of the pumping pulse. The main decay of the ESE signal occurs within the characteristic time $T \sim 1/D$ where D is a typical value

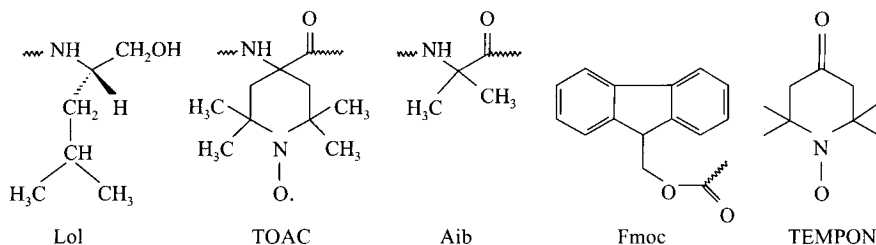


Fig. 1. The structures of Lol, TOAC, Aib, Fmoc and TEMPON.

of the dipole-dipole spin coupling between spins A and B. In conventional experiments, the time interval τ between the first and second pulses is fixed and the signal decay at time T , $V(T)$ is analyzed.

When spins are randomly distributed in solids, the relaxation of transverse magnetization due to spin-spin dipole coupling of spins in the bulk, $V(T)$, is described by the conventional exponential law with time [4]. The relaxation time, in this case, depends on the value of dipole-dipole interaction of spins and their concentration. Deviations from the random distribution of spins lead to the deviations from exponentiality of the PELDOR decay $V(T)$. For example, when the local concentration of spins exceeds the mean value (spin pairs, groups, clusters, etc.), at short times there is a more effective relaxation owing to strong dipole-dipole interaction of spins at short distances. As a result, there is a fast decay of PELDOR signal at short times corresponding to the strong dipole-dipole interaction of spins in clusters. When the time T increases, relaxation effectiveness becomes weaker and approaches the relaxation effectiveness of the random spin distribution in the system under investigation [4].

In the case of a fixed distance R between spins (biradicals, spin pairs, double-labeled peptides) the relaxation decay of PELDOR signal is accompanied by oscillations at frequencies $\omega \sim 1/R^3$. In this case it is possible to obtain the information about distances in the spin system [4].

It was found for peptides III and V [3] that the oscillation frequency and amplitude of PELDOR signal decay strongly depend upon the position of spin labels in the peptide structure and the difference in the structure of terminal peptide groups which could affect the structure of forming aggregates. These preliminary observations [3] required the additional investigations of PELDOR relaxation effects in spin-labeled peptide aggregates changing the structure of peptides, position of label and the properties of solvents (polarity, ability to complex formation, etc.). Starting this program we have studied the dipole-dipole relaxation of spin labels for peptides I–VII in different weakly polar solvents. The structure of the peptides under study is shown in Table 1. Peptide I containing two spin labels was used in test measurements of experimental parameters on the basis of the study of the intramolecular dipole-dipole interaction of its two spin labels. The solution of nitroxyl radical TEMPO in a chloroform-toluene mixture was used as an example of the random distribution of spins in solid glass.

2 Experimental

The cw ESR spectra of labeled peptides under study were recorded on an ESP-380 Bruker spectrometer at a modulation frequency of 100 kHz and a modulation amplitude of 0.1 mT in the absence of spectrum saturation.

Experiments on PELDOR were carried out on an X-band ESE spectrometer supplied with a bimodal resonator and a device for generating pumping pulses [5]. The registration frequency of spectrometer ω_A was 9476 MHz. The difference in registration and pumping frequencies ($\omega_A - \omega_B$) was about 100 MHz. Dura-

tions of the first and second mw pulses forming spin echo were 40 and 70 ns, respectively. The duration of the pumping mw pulse was about 40 ns.

Seven synthetic peptides (Table 1), wherein Aib has been substituted by TOAC, were used in the experiments. It has been shown before that the replacement of an Aib by a TOAC residue does not influence either the peptide conformation or the membrane-modifying properties. In peptides I–VII the C-terminal leucinol of trichogin GA IV has been replaced by leucine methyl ester (Leu-OMe), and in peptides I–IV, the N-terminal *n*-octanoyl group has been substituted by the Fmoc-protecting group. A change of the N- and C-terminal groups does not influence the peptide conformation, but the membrane-modifying properties are modified by the substitutions [6, 7].

The samples (glass ampoules with a diameter of about 0.5 cm) contained about 0.1 ml of the solution. As a solvent for peptides II–VII and TEMPON, we used a chloroform-toluene mixture in a 1:1 ratio by volume. In addition, to elucidate the influence of solvent properties, the solutions of peptide VI were also studied in mixtures of chloroform-decalin in a 1:1 ratio, 1,2-dichloroethane-toluene in a 1:2 ratio and tetrachloromethane-toluene in a 1:2 ratio by volume. To avoid the aggregation [3], we have studied solutions of peptide I in polar solvent ethanol. The samples were frozen by inserting the ampoules into liquid nitrogen. After freezing, the mixtures took the form of a transparent glassy mass. The chemically pure solvents were used without additional purification. Experimental conditions differed from those used previously [3] by solvent composition and a wider time T domain which allowed us to study in more detail the peculiarities of the PELDOR relaxation decay $V(T)$. For comparison under the same conditions, the data are given for peptides III and V studied previously [3].

To obtain cw ESR spectra and PELDOR data, the samples were located in the pin of a Dewar flask cooled by liquid nitrogen and placed in the spectrometer resonator. The number of spin labels in the samples was determined by comparing the double integrals of ESE spectra with the similar values for $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ crystals containing the known number of paramagnetic centers. The concentration of spin labels in the samples under study was about $2 \cdot 10^{18} \text{ cm}^{-3}$ and was not varied during experiments.

3 Results and Discussion

Figure 2 shows the cw ESR spectra of frozen glassy solutions of spin-labeled peptides I–VII and the spectrum of a stable nitroxyl radical TEMPON. The spectrum of double-labeled peptide I was obtained for the solution of this peptide in ethanol. The cw ESR spectra of peptides II–VII and radical TEMPON were obtained for their solutions in the chloroform-toluene mixture in a 1:1 ratio by volume. Similar spectral shapes are observed for the cw ESR spectra of peptide VI in the glassy mixtures of chloroform-decalin 1:1, dichloroethane-toluene 1:2, tetrachloromethane-toluene 1:2. A comparison with TEMPON shows that these peptide spectra are typical for nitroxyl radicals in frozen glassy solutions. The dipole-dipole interaction between spin labels is masked by the inhomogeneous

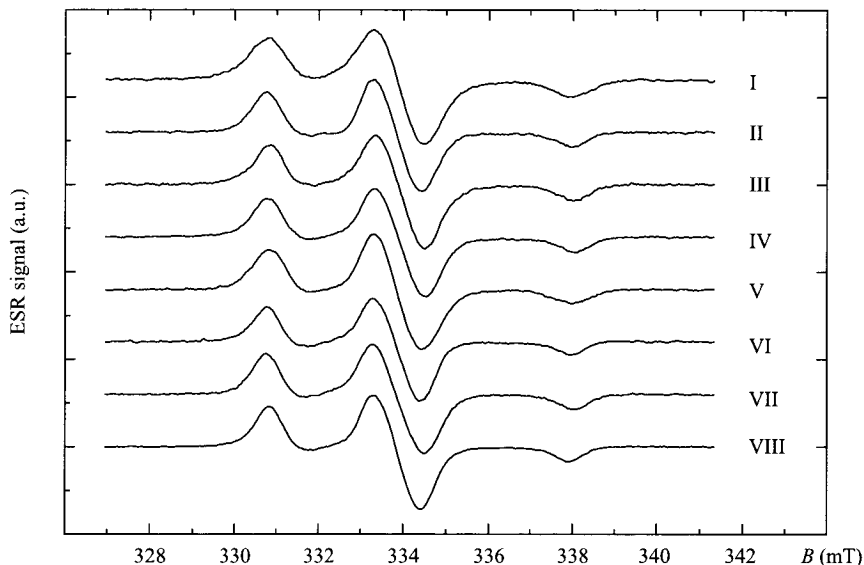


Fig. 2. ESR spectra of peptides I–VII and TEMPON in glassy solutions at 77 K. The spectrum number for peptide in the figure coincides with that in Table 1. I, peptide I in ethanol; II–VII, peptides II–VII in chloroform-toluene mixture (1:1); VIII, TEMPON in the same mixture.

broadening of spectra and cannot be obtained by analyzing the width and shape of cw ESR spectra. The spectra of the double-labeled peptide I actually coincide with those of the mono-labeled peptides II–VII, the dipole-dipole interactions between two labels is weak and also masked by inhomogeneous broadening of the lines. This makes it possible to use the solutions of peptides I for experimental determination of the value of probability of spin B flip by the pumping pulse, p_b , which is important for analysis.

Figure 3 shows the log plot of the PELDOR signal amplitude V versus position of the pumping pulse at time T for frozen glassy solutions of TEMPON and peptides I–VII. Curve a refers to the solution of TEMPON in the chloroform-toluene mixture. In this case, we observe the exponential decay (linear dependence of $\ln V$ on T) typical for the dipole-dipole relaxation of the randomly distributed spins [4]. Curve I belongs to the solution of the double-labeled peptide I in ethanol. Curves II–VII refer to the solutions of peptides II–VII in the chloroform-toluene mixture. As compared with curve a, curves I–VII display a fast decrease in the PELDOR signal at short times T (up to 150 ns) with subsequent slow decay followed, in some cases, by PELDOR signal oscillations with time T . A fast decrease in the PELDOR signal at short times for peptide I containing two spin labels is determined by the intramolecular dipole-dipole coupling of these labels. These types of PELDOR decay were repeatedly recorded previously in solid glasses for biradicals and similar peptides containing two spin labels [1–4].

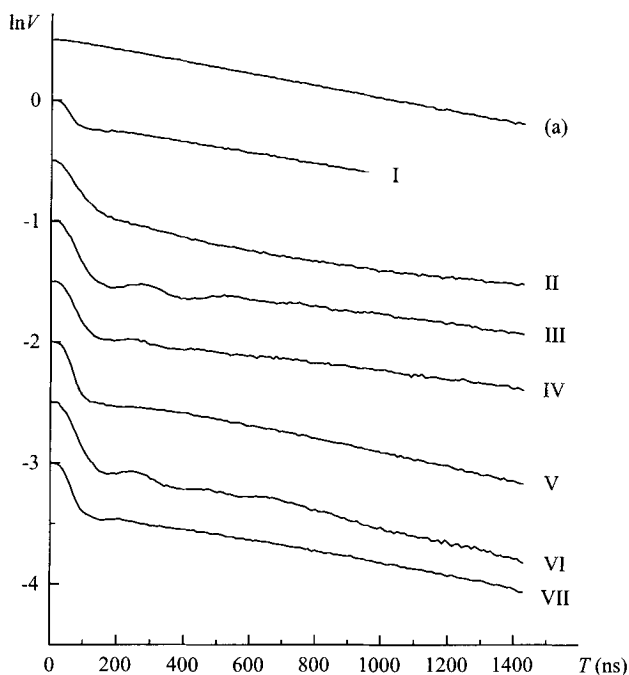


Fig. 3. PELDOR signal decay for glassy solutions of peptides I–VII and TEMPON at 77 K. The curve number for peptide in the figure coincides with that in Table 1. a, TEMPON in chloroform-toluene mixture; I, peptide I in ethanol; II–VII, peptides II–VII in chloroform-toluene mixture (1:1).

Curves of the II–VII type for PELDOR decay (Fig. 3) were observed for the mono-labeled peptides II and V in the chloroform-toluene mixture of a 7:3 ratio [3]. According to [3], a fast decrease in PELDOR signal at short T times (up to 150 ns) for curves II–VII indicates the formation of aggregates from mono-spin-labeled peptides. A strong dipole-dipole interaction of spin labels in the aggregates leads to fast decay of the PELDOR signal during the initial time period followed by oscillations caused by peptides in the aggregates with a mutual regular arrangement of spin labels (a case of fixed distances [4]). A further decrease in PELDOR signal is caused by the dipole-dipole interaction of labels among different aggregates. According to [3, 4], in general, the PELDOR signal dependence on time $V(T)$ can be given as a product of two terms: $V(T)_{\text{intra}}$, the decay function due to dipole-dipole couplings between labels inside aggregates, and $V(T)_{\text{inter}}$, the same for coupling of labels between aggregates:

$$V(T) = V(T)_{\text{intra}} V(T)_{\text{inter}}. \quad (1)$$

This representation holds for the case of independent intra- and interaggregate couplings of labels.

Information about the aggregate structure is contained in the PELDOR signal dependence on time owing to the coupling of labels in the aggregates, $V(T)_{\text{intra}}$. According to [4], the dependence of $V(T)_{\text{intra}}$ on time T can be represented as

$$V(T)_{\text{intra}} = \frac{1}{N} \sum_{j=1}^N \left(\prod_{\substack{k=1 \\ k \neq j}}^N \{1 - p_b [1 - \langle \cos(D_{jk}T) \rangle]\} \right), \quad (2)$$

$$D_{jk} = \frac{\gamma^2 \hbar (1 - 3 \cos^2 \theta_{jk})}{r_{jk}^3}, \quad (3)$$

where $V(T)_{\text{intra}}$ is the signal amplitude normalized to unity at $T = 0$, T is the time interval between the first pulse at ω_A and the pumping pulse at ω_B , N is the number of spin labels in the aggregate, j is the number of spins A in an aggregate, k is the number of spins B, p_b is the probability of spin B flip by the pumping pulse, γ is the gyromagnetic ratio for an electron, \hbar is the Planck constant, r_{jk} is the distance between spins j and k , θ_{jk} is the angle between vector r_{jk} and external magnetic field, $\langle \rangle$ is the averaging over possible values r_{jk} and θ_{jk} .

Averaging over angles θ_{jk} in Eq. (2) owing to the unavoidable random orientation of aggregates in a glassy solution provides a fast decrease in echo signal at times T corresponding to the value of the mean dipole-dipole interaction of labels inside the aggregate with subsequent small oscillations at frequencies ω_{jk} :

$$\omega_{jk} = \frac{\gamma^2 \hbar}{r_{jk}^3}. \quad (4)$$

In addition, the averaging over angles in Eq. (2) leads to the oscillation amplitude damping with increasing time T . In this case, the $V(T)_{\text{intra}}$ value tends to its limiting value V_p , oscillating around it with frequencies according to Eq. (4). The V_p value is found from Eq. (2) at $\langle \cos(D_{jk}T) \rangle = 0$:

$$V_p = (1 - p_b)^{N-1}. \quad (5)$$

As time increases, the oscillations can attenuate due to the averaging of Eq. (2) over both the angles and the distances between spin labels with a spread in r_{jk} . As a result, the $V(T)_{\text{intra}}$ value becomes time-independent. Such behavior of $V(T)_{\text{intra}}$ with time makes it possible to divide the contributions of the interaction of labels inside and between aggregates into the PELDOR signal in terms of Eq. (1). To this end, after the initial sharp decay, the experimental dependences $\ln V(T)$ in Fig. 3 were considered to decrease due to the interaggregate interaction of labels and represented by a second-order polynomial in the form of a smooth nonoscillating curve $\ln V(T)_{\text{inter}}$. Subtracting the resulting dependence $\ln V(T)_{\text{inter}}$ from the total dependence $\ln V(T)$, we get the dependence $\ln(V/V_{\text{inter}})$ close to the

intraaggregate $\ln V_{\text{intra}}$ and sufficient to estimate oscillation frequencies and V_p values. Figure 4 shows the dependences of the PELDOR signal decay on time T owing to the interaction of spin labels in the aggregates $\ln(V/V_{\text{inter}})$ for peptides I–VII in the chloroform-toluene mixture obtained in this manner from the curves I–VII in Fig. 3. The data extracted by a similar procedure for peptide VI in other solvents are given in Fig. 5.

The oscillations of V_{intra} on time T in Figs. 4 and 5 indicate that the aggregates include fragments with a fixed structure in which the distances between spin labels are determined with a minor spread. In this case the mean distances r between spin labels in these fragments can be estimated from the experimental oscillation frequencies by the frequency-distance relation Eq. (4). The oscillation frequencies and the corresponding mean distances r are presented in Table 2 along with the V_p values measured by the dependences in Figs. 4 and 5 and the mean quantities of labels in aggregate N calculated from Eq. (5). N was determined for all peptides with $p_b = 0.17$ calculated from Eq. (5) for peptide I containing two spin labels and serving as the reference specimen. Note that the method used to share out the interaggregate term V_{intra} from the general signal decay function $V(T)$ could be used only in the case if V_{intra} decays fastly (at short

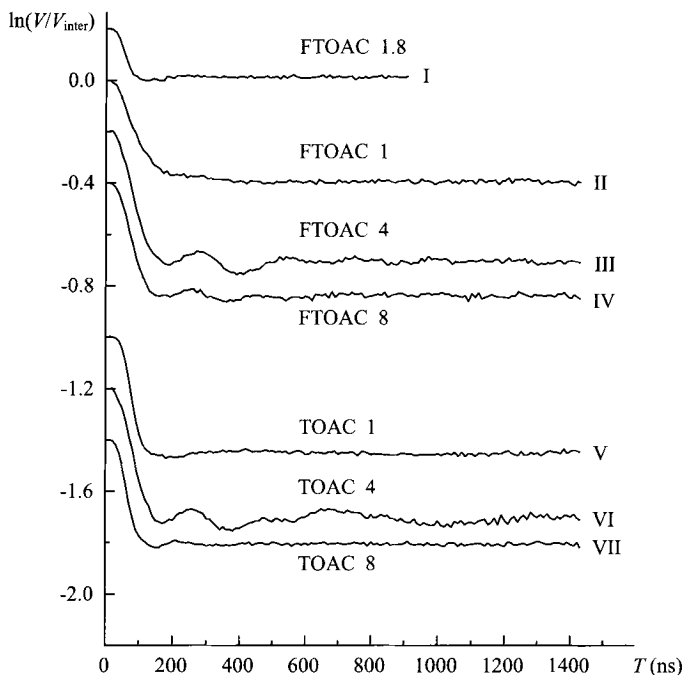


Fig. 4. PELDOR signal decay due to interaction of labels in aggregates for glassy solutions of peptides I–VII in a frozen mixture of chloroform-toluene. The curve number for peptide in the figure coincides with that for peptide in Table 1. I, peptide I in ethanol; II–VII, peptides II–VII in chloroform-toluene mixture.

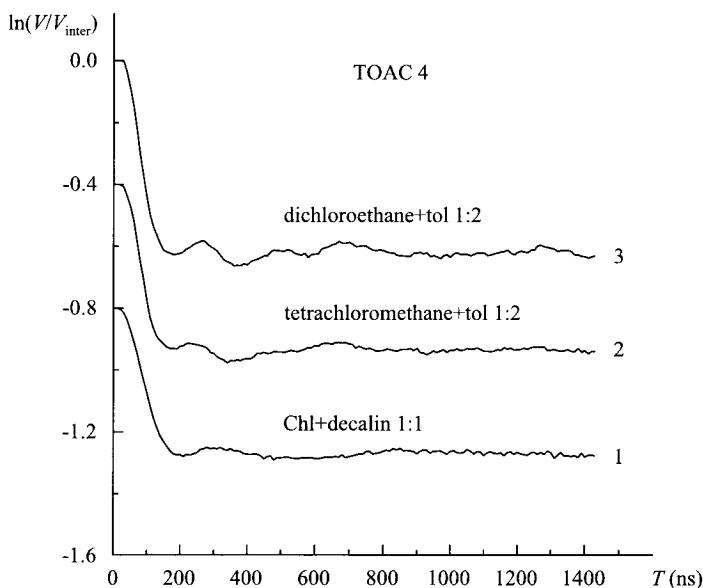


Fig. 5. PELDOR signal decay due to interaction of labels in aggregates for glassy solutions of peptide VI in the following solvents: 1, the mixture of chloroform with *cis-trans* decalin 1:1 by volume; 2, the mixture of tetrachloromethane with toluene 1:2 by volume; 3, the mixture of dichloroethane with toluene 1:2 by volume.

Table 2. Experimental frequencies of oscillations, distances between spin labels r_{jk} , V_p and N values for different spin labeled peptides.

Peptide		Solvent (volume mixtures)	Frequency (MHz)	r_{jk} (nm)	$V_p \pm 2\%$	$N \pm 4\%$
I	FTOAC 1.8	ethanol	—	—	0.17	2
II	FTOAC 1	chloroform+toluene (1:1)	—	—	0.67	3.14
III	FTOAC 4	chloroform+toluene (1:1)	3.85 ± 0.15	2.37 ± 0.03	0.6	3.75
IV	FTOAC 8	chloroform+toluene (1:1)	5.0 ± 0.25	2.18 ± 0.036	0.64	3.4
V	TOAC 1	chloroform+toluene (1:1)	2.5 ± 0.3	2.74 ± 0.11	0.63	3.5
VI	TOAC 4	chloroform+toluene (1:1)	4.3 ± 0.2 1.5 ± 0.2	2.29 ± 0.035 3.25 ± 0.14	0.6	3.75
VI	TOAC 4	chloroform+ <i>cis-trans</i> decalin (1:1)	3.3 ± 0.2 1.2 ± 0.2	2.50 ± 0.05 3.50 ± 0.19	0.62	3.56
VI	TOAC 4	CCl_4 +toluene (1:1)	4.3 ± 0.2 1.5 ± 0.2	2.29 ± 0.035 3.25 ± 0.14	0.58	3.92
VI	TOAC 4	$ClCH_2CH_2Cl$ +toluene (1:2)	4.3 ± 0.2 1.5 ± 0.2	2.29 ± 0.035 3.25 ± 0.14	0.54	4.3
VII	TOAC 8	chloroform+toluene (1:1)	4.5 ± 0.2	2.26 ± 0.033	0.66	3.23

T) to its limiting value V_p , which is a function independent of T . In order to prove it, we studied the concentration dependence of $V(T)$ for spin-labeled peptide II in chloroform-toluene (1:1). In this case extrapolating the dependence $V(T)$ upon the concentration of labels (C_R) in solution to $C_R = 0$ it is possible to obtain a more accurate shape of V_{intra} vs. T [8]. With this approach we found that the V_p value can be found in this experiments at $T > 250$ ns. It does not contradict our results obtained earlier by a much more simple way.

The dependences shown in Fig. 4 for peptides II–VII differ in the type of terminal group and the position of spin label in peptide structure. Curves II–IV refer to peptides containing Fmoc as an N-terminal group, curves V–VII refer to the peptides with the N-terminal *n*-octanoyl group. As follows from Fig. 4, the structure of the terminal group has a weak effect on $V(T)$ whereas the oscillation amplitude and frequency substantially depend on the position of spin label in the peptide structure. Note that the oscillation amplitude and frequency measured for peptides III and V are close to those given earlier [3]. A minor difference in them can be assigned to the different compositions of solvents.

Of interest is the existence of two oscillation frequencies in the case of peptide VI presented in Table 2. This can testify to both the existence of two fixed distances inside the aggregate and a possible existence of the two aggregates of different types with different distances between spin labels. Unfortunately, the data available do not allow us to choose between these possibilities.

The dependences given in Figs. 4 and 5 for peptide VI indicate that the aggregate properties depend on the solvent nature. It appears from the figures that all parameters, such as frequency, amplitude and oscillation damping speed, change when one solvent of the binary solvent mixture is changed to another. This is best observed when comparing the $V(T)$ decay for peptide VI solutions in the chloroform-toluene (Fig. 4, curve VI) and chloroform-decalin (Fig. 5, curve I) mixtures. Substituting toluene by decalin, the oscillation frequencies decrease and the oscillation damping speed increases which can be assigned to some “loosening” of aggregates and disorder in their structure. It is noteworthy that despite some differences in the amplitudes and oscillation damping speed depending on peptide structure and solvent properties, the distances between spin labels (Table 2) in aggregates are grouped around the values of 2.3, 2.6 and 3.3 nm.

It is worthwhile to underline that the use of experimental V_p values for determining the number of labels in aggregates from Eq. (5) gives the correct number of labels in the aggregate providing that aggregates in solution contain the identical number of labels. In the case of distribution in the number of labels within the population of aggregates or in the presence of some fraction of monomeric peptide molecules, the V_p value depends not only on the parameter of the pumping pulse p_b but also on the similar parameter p_a of pulses forming echo signal [4, 9]. The effective number of N found from the experimental V_p value will be in this case lower than the true mean number of labels in aggregates. In this case, the parameters of the distribution in the number of labels in aggregates can be estimated by the approach given in [4, 9]. This approach will necessitate additional measurements with changes of the p_b and p_a parameters.

The values of the effective number of spin labels N in aggregates (Table 2) vary from 3.1 to 4.3 depending on the peptide structure and solvent composition. As mentioned above, this distribution in the number of labels can be related to the distribution in the number of labels in aggregates or the presence of the uncontrolled number of unbound peptide molecules in solution. A change in these parameters with peptide structure and solvent composition can lead to the corresponding changes in the N value determined from Eq. (5). Besides, a certain error in the measurement of N can be caused by differences between the p_b values of the peptides studied and p_b for peptide I chosen as a reference with $N = 2$. These differences can arise from the fact that the p_b value is the convolution of the absorption line shape of the ESR spectrum with the pumping pulse parameters [4] and the deviations of the determined value of N from its true value can be due to the slight differences in ESR spectra. Therefore we have at least two reasons for the obtained spread in N values.

Thus, the study of the spin-labeled analogs of trichogin shows that the formation of aggregates in weakly polar solvents is typical for these molecules. The aggregate structure mainly depends on solvent composition and, to a lesser degree, on the type of terminal groups. Estimating the distances between spin labels in aggregates from oscillation frequencies shows that the distances between the labels group, with a small spread, around the values of 2.3, 2.6 and 3.3 nm. Estimation of the number of spin labels in aggregates on the basis of the experimental data on the behavior of PELDOR signal at the long time interval gives the lower boundary of the number of peptide molecules in aggregates to within 3.1–4.3. These data can serve as a basis for improving the spacial arrangement of peptide molecules in the cluster that has been proposed in [3].

4 Conclusions

The method of pulsed double resonance in electron spin echo combined with the method of spin labels was used to study the peculiarities of the self-aggregation of trichogin analogs in weakly polar solvents. The dipole-dipole spin-spin relaxation of labels was experimentally studied in aggregates in glassy peptide solutions frozen to 77 K depending on peptide structure and solvent composition. It is shown that the formation of aggregates in weakly polar solvents is typical for the trichogin analogs. The aggregate structure depends on solvent composition and the structure of terminal groups of peptides. It has been established that the distances that can be measured between spin labels in aggregates group around the values of 2.3, 2.6 and 3.3 nm. The lower boundary has been experimentally obtained for the mean number of peptide molecules in aggregates in the range of 3.1–4.3 molecules depending on peptide structure and solvent composition.

Acknowledgements

We are extremely grateful to Prof. Dr. C. Toniolo and his collaborators Dr. F. Formaggio and Dr. M. Crisma (Biopolymer Research Centre, CNR, Department of

Organic Chemistry, University of Padova, Padova, Italy) for their gift of the spin-labeled peptides. This work was supported by The Netherlands Organisation for Scientific Research (NWO) project 047.006.009, CRDF grant 6350 and partly by the Russian Basic Research Foundation, grants 95-03-10770, 99-03-33149, 00-15-97321.

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