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SOLVENT-DEPENDENT STRUCTURAL FEATURES OF THE MEMBRANE ACTIVE PEPTIDE TRICHOTOXIN A40 AS REFLECTED IN ITS DIELECTRIC DISPERSION

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The dielectric dispersion of trichotoxin A40 in solvents of diverse lipophilicity (i.e. *n*-octanol, dioxane and mixtures of these) has been measured between 100 kHz and 50 MHz. In pure octanol also the weight average molecular weight and the concentration dependence of the circular dichroism spectrum were determined. An analysis of the data leads to the conclusion that in octanol an appreciable amount of the solute exists as single monomeric particles which are about 30 Å long and 12.5 Å in diameter. There is a dipole moment parallel to the long axis. In addition the evidence points at the presence of small aggregates (mainly dimers and trimers) formed by some kind of head-to-tail association. Evidently dioxane added to octanol favors a different structure of the trichotoxin monomer. It is apparently involved in another aggregation process resulting in aggregates of large size but comparatively low dipole moments.

1. Introduction

Certain polypeptide antibiotics are known to cause voltage-dependent pore formation in lipid bilayer membranes. Among these agents, alamethicin F30/50 has been studied most extensively [1–4]. A sequentially similar peptide, trichotoxin A40/50 [5], is another member of this group [6]. (For sequences, see p. 420.)

The natural analogues alamethicin F50 and trichotoxin A50, respectively, have a glutamine residue instead of the glutamic acid at the C-terminal side.

Alamethicin and trichotoxin differ in their pore-forming properties. Both induce a voltage-dependent electrical conductivity for the sequentially shorter trichotoxin, however, resolvable pore states could not be recorded [7]. Recently stable conductance levels were obtained upon attaching to its C-terminus a large polar residue such as the dansyl group [8].

The membrane modifying action in question leads to ionic permeability changes and electrical excitation which are phenomenologically equivalent to those observed with nerve membranes. The underlying molecular mechanism of such biologically important processes is so far not clearly understood. In this regard it is of particular interest to explore in which way the electric field in the membrane may directly influence pertinent functional steps. Basic information needed apparently concerns possible molecular structures and the electric dipole moments associated with them.

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Alamethicin F30 (molecular mass 1964):

Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phe-OH
(Aib)

Trichotoxin A40 (molecular mass 1704):

Ac-Aib-Gly-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Aib-Aib-Pro-Leu-Aib-Iva-Glu-Val-OH
(Ala) (Ala) (Aib)

According to circular dichroism and NMR studies the N-terminal regions of the peptides under consideratoin adopt α-helical conformations. These are, however, comparatively short in hydrophilic solvents but become much larger in more lipophilic ones [7,9–12] suggesting a conformational transition upon a change of the environments. There is also a tendency to form oligomeric aggregates. It may involve antiparallel arrangement of α-helic dipoles as indicated by the X-ray structure of an undecapeptide model of the alamethicin helix [13]. On the basis of an electric field induced flip-flop of such α-helix dipoles one can explain the voltage-sensitive pore formation [14].

An alternative to be considered is the possibility that the field causes a transition between structural states which have different pore forming activities [15,16]. In fact, any conformational equilibrium would be displaced by an applied electric field if only the structural transformation leads to an enhanced dipole moment (parallel to the field) [17].

Appropriate data regarding structural and dipolar properties can be obtained by means of dielectric dispersion measurements. A pertinent study has already been carried out with alamethicin in solvents of various degrees of lipophilicity, namely n-octanol and dioxane as well as mixtures of these [18,19]. The results indicate that in pure octanol a highly dipolar monomeric structure predominates which has a fairly elongated shape. On the other hand, addition of dioxane was found to favor less polar and rather large aggregates of the peptide.

In the present article we report on an analogous investigation with trichotoxin A40 in the same solvents.

2. Material and Methods

Trichotoxin A40 was prepared as described elsewhere [20]. Preparing solutions and performing measurements has been done in the same way pursued in the recent experiments with alamethicin [19].

Thus the dielectric increment, $\Delta\varepsilon'$ (i.e., the difference between the dielectric constants of the solution and the solvent), as well as the dielectric loss, ε'', were determined in the frequency range from 5 kHz up to 50 MHz. On the basis of conventional Cole-Cole plots these data could be well fitted to the appropriate relation

$$\Delta\varepsilon' - i\varepsilon'' = \frac{\Delta\varepsilon_0}{1 + (if/f_0)^{1-\alpha}} + \Delta\varepsilon_\infty \qquad (1)$$

(where $i = \sqrt{-1}$; f, frequency of the applied electric field; $\Delta\varepsilon_\infty$, high frequency limit of $\Delta\varepsilon'$). This yields three phenomenological parameters of dielectric dispersion: the total dielectric increment in the limit of low frequencies, $\Delta\varepsilon_0$; the dispersion frequency, f_0; and a quantity reflecting the width of a relaxation spectrum, α. Concentrations of the peptide have been calculated from the amounts weighed in solution. This was confirmed by checking the circular dichroism spectra (recorded with a Cary 61 instrument); appropriate values of molar ellipticity were determined with standards using amino acid analysis to verify the concentration.

3. Experimental Results

Fig. 1 presents a typical dispersion curve measured with trichotoxin in pure octanol. At frequencies below 100 kHz an additional increase of $\Delta\varepsilon'$ of substantial magnitude has been observed just as

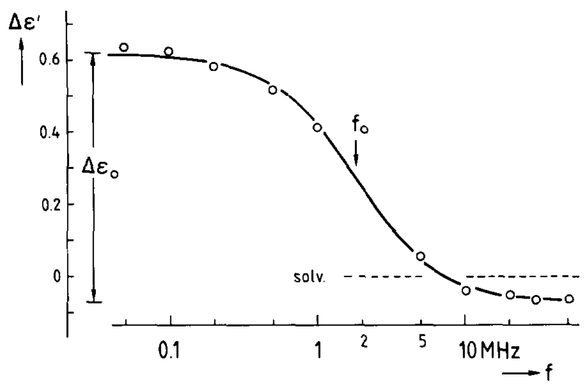

Fig. 1. Dielectric increment $\Delta\varepsilon'$ of trichotoxin in pure octanol as a function of frequency ($C_0 = 4$ mg/ml, 25°C). The solid curve was calculated for a Cole-Cole relation according to Eqn. 1 with $\Delta\varepsilon_o = 0.69$, $f_o = 1.8$ MHz, $\alpha = 0.1$, $\Delta\varepsilon_\infty = -0.07$. The dashed line indicates the level of the pure solvent (i.e., $\Delta\varepsilon' = 0$).

encountered with alamethicin [19]. Again we noted that it does not occur in the more dioxane enriched solvent mixtures. The nature of this peculiar low frequency effect is not clear so far. For the present we leave it out of our considerations.

In contrast to alamethicin we have found that trichotoxin undergoes an appreciable dielectric dispersion even in pure dioxane. A measured example is shown in Fig. 2 (though with the peptide concentration definitely greater than in the case of Fig. 1).

Under various conditions of solvent composition, peptide concentration and temperature dispersion curves were evaluated regarding the dielectric parameters of the Cole-Cole approach.

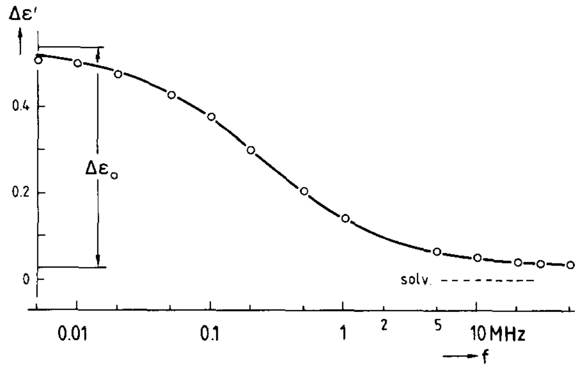

Fig. 2. Dielectric increment $\Delta\varepsilon'$ of trichotoxin in pure dioxane plotted analogously as the data of Fig. 1 ($C_0 = 11$ mg/ml, 25°C; $\Delta\varepsilon_o = 0.51$, $f_o = 0.23$ MHz, $\alpha = 0.33$, $\Delta\varepsilon_\infty = +0.03$).

The dispersion frequency, f_0, as it resulted at 25°C for a number of different values of the octanol content is given in the diagram of Fig. 3a (no significant effect of the peptide concentration is expected and was in fact not observed). We may assume an ordinary rotational mechanism of dielectric polarization so that f_o is determined by an appropriate (mean) rotary frictional coefficient of the dipolar particles. Accordingly it should be inversely proportional to the viscosity of the solvent, η, provided the structure of these particles does not change. Relative to the case of pure octanol the f_o should then follow the course of the dashed curve when the respective values of η are taken into account as measured previously [19]. Evidently, however, the actual dispersion frequen-

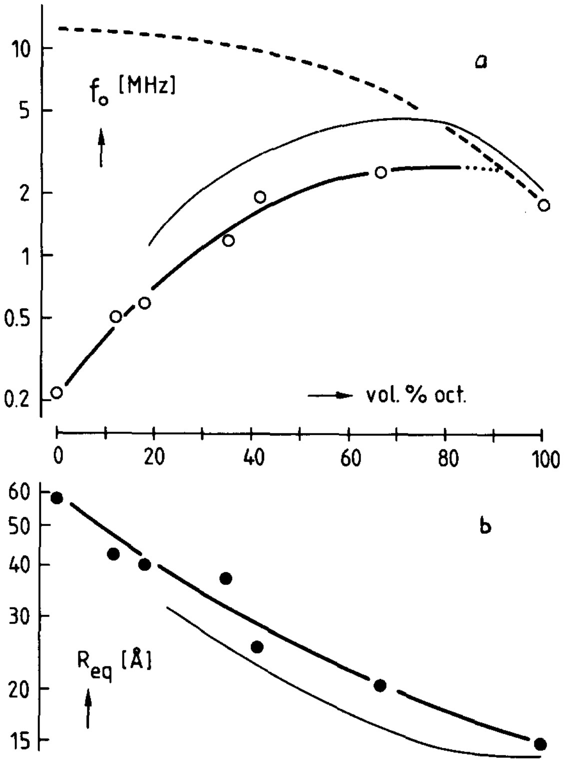

Fig. 3. (a) Dispersion frequency f_o as measured for various octanol contents of the solvent (at 25°C). The dashed curve has to be expected if only the effect of solvent viscosity would be responsible for the change of f_o (relative to the pure octanol case). (b) Radius of an equivalent spherical particle calculated by means of Eqn. 2. Thin lines represent data previously obtained for alamethicin [19].

cies become much smaller once the octanol content in dioxane decreases. They apparently fall somewhat below those for alamethicin (indicated by the faint curve) but qualitatively the dependence on solvent composition is fairly much the same.

This behavior of f_o can be interpreted as being caused by a gradual increase of the average dimensions of the dipolar particles. Formally one may express it in terms of an equivalent radius [19]

$$R_{eq} = \left\{ kT / \left(8\pi^2 \eta f_o \right) \right\}^{1/3} \tag{2}$$

(k, Boltzmann's constant; T, absolute temperature) defined by spherical particles of uniform size which would exhibit the given f_o. From our data we have calculated the equivalent radii in Fig. 3b. They refer to a temperature of 25°C. Measurements at 40°C and, in some cases, at 10°C have led to considerable changes of f_o but no significant variation of $\eta f_o / T$ so that the R_{eq} remains practically invariant. In other words, the temperature has no apparent effect on particle size and shape. The total dielectric increment $\Delta\varepsilon_0$ naturally depends on the peptide concentration. Values determined for a 4 mg/ml solution at 25°C are given in Fig. 4a. Most of them have actually been measured at this concentration, only two (namely for 41.5 vol.% octanol and one for pure dioxane) result from experiments with higher concentrations of trichotoxin and have been appropriately corrected assuming a linear concentration dependence. The effect of solvent composition on the spectral distribution parameter α is represented in Fig. 4b.

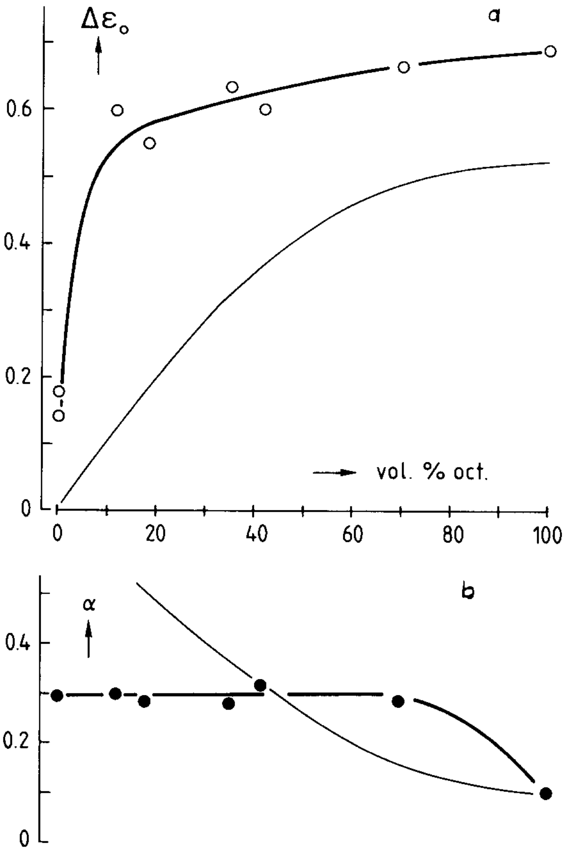

Fig. 4. Dependence on solvent composition as reflected by (a) the total dielectric increment $\Delta\varepsilon_0$ (see text) and (b) the spectral distribution parameter α. The faint curves again indicate the respective results for alamethicin [19].

According to our measurements of $\Delta\varepsilon_0$ at different temperatures there is no significant change of the dipole moment. On the other hand, elevation of temperature as well as peptide concentration induced slight increases of the parameter α. In addition we have carried out some experiments providing useful information regarding particle size and structure. From sedimentation equilibrium in the analytical ultracentrifuge the weight average molecular weight, M_w, of the trichotoxin solute in pure octanol (at 4 mg/ml, 25°C) was determined to be about 3400 which is practically twice the monomer molecular weight, $M_1 = 1704$. This indicates a somewhat higher degree of aggregation than encountered for alamethicin under the same circumstances [19]. Furthermore, examination of the circular dichroism spectrum up to 1.6 mg/ml revealed an exact linear dependence on concentration. Hence we may exclude the possibility that aggregation induced by a concentration increase (in the range of a few mg/ml) is associated with a change of secondary structure of the trichotoxin particles.

4. Discussion

4.1. Aggregation pattern in octanol

Because of $M_w/M_1 \approx 2$ a considerable extent of aggregation of trichotoxin in octanol has to be taken into account. The definite distribution of aggregates expressed as respective mass fractions is an important basic point in a proper approach to analyse the dielectric data obtained in the experiments.

We shall inspect first the possibility of micelle formation, namely that essentially only one aggregate of n monomers is formed. Regarding the mass fractions y_1 and y_n of the monomer and the n-mer, respectively, we then have the equations

$$y_1 + y_n = 1, \quad y_1 + ny_n = M_w/M_1 = 2$$

leading to

$$y_1 = (n-2)/(n-1); \quad y_n = 1/(n-1) \tag{3a,b}$$

Thus $y_1 = 0$ in case of $n = 2$, i.e. dimer micelles would exist as a practically sole particle type in the system. The larger the micelle, however, the more monomers must coexist ($y_1 = 0.5$ for $n = 3$, $y_1 = 0.67$ for $n = 4$, and so on).

Turning now to the most complex scheme of aggregation involving any aggregate of n monomers, A_n, we note that a corresponding series of individual aggregation steps

$$A_n + A_1 \rightleftharpoons A_{n+1} \quad \text{for } n = 1, 2, \ldots \tag{4a}$$

would exist, each described by a respective equilibrium constant K_n. Naturally this comprises an infinite number of different possibilities. Under the special circumstances of our trichotoxin/octanol system we have

$$\Sigma_n y_n = 1, \quad \Sigma_n ny_n = M_w/M_1 = 2 \tag{4b,c}$$

Accordingly monomers must always be present apart from the trivial dimeric micelle case mentioned already.

The binding affinity of A_1 to any A_n may be the same so that $K_n = K$ is independent of n. This case is known as isodesmic aggregation. As can easily be derived, it implies

$$y_n = ns^{n-1}(1-s)^2 \tag{5a}$$

involving a parameter s (< 1) subject to the relation

$$s/(1-s)^2 = KC_0/M_1 \tag{5b}$$

(C_0, total concentration of solute as mass per volume).

Furthermore one finds

$$M_w/M_1 = (s+1)/(s-1) \tag{5c}$$

Assuming that this case applies to our system we obtain $s = 0.33$ and accordingly $y_1 = 0.45$, $y_2 = 0.30$, $y_3 = 0.15$ leaving a total mass fraction of 0.1 for all larger aggregates.

4.2. Analysis of dispersion curves

The measured frequency dependence of $\Delta\varepsilon' - \Delta\varepsilon_\infty$ can be regarded as a superposition of so-called Debye-functions represented by individual terms

$$\Delta\varepsilon_{oj}/\left\{1 + (f/f_{oj})^2\right\} \tag{6}$$

Each running number j reflects a separate dielectrix relaxation step with a respective total dielectric increment $\Delta\varepsilon_{oj}$ and dispersion frequency f_{oj}.

Let us approximate the solute particles as spheroids (i.e. ellipsoids of revolution) with a, b being the semi-axes in the direction of symmetry and perpendicular to it, respectively. Then each particle type can contribute one Debye term which is associated with the parallel dipolar component and another one determined by the perpendicular component. The individual f_{oj} may be expressed [19] as

$$f_{oj} = \frac{RT}{6\pi\eta\gamma_s nM_1\bar{v}}\phi_j(p) \tag{7}$$

(R, gas constant; n, aggregation number; $\bar{v}$, partial specific volume of the solute; $p = a/b$). The factor γ_s stands for a factor correcting the apparent particle volume (from $\bar{v}$) to yield the true volume (which may be somewhat different due to solvation effects). The various possible functions $\phi_j(p)$ result from hydrodynamic theory. They are graphically presented in Fig. 5. For trichotoxin in octanol we put $\bar{v} = 0.80$ ml/g as measured for alamethicin [19]. At 25°C Eqn. 7 then yields

$$\phi_j(p) = 0.09n\gamma_s(f_{oj}/\text{MHz}) \tag{8}$$

This can be used to determine p from a known f_{oj}. Thereafter the two semi-axes a and b are available separately by taking advantage of the fact that the particle volume equals $(4\pi/3)a^3p^{-2}$. Generally

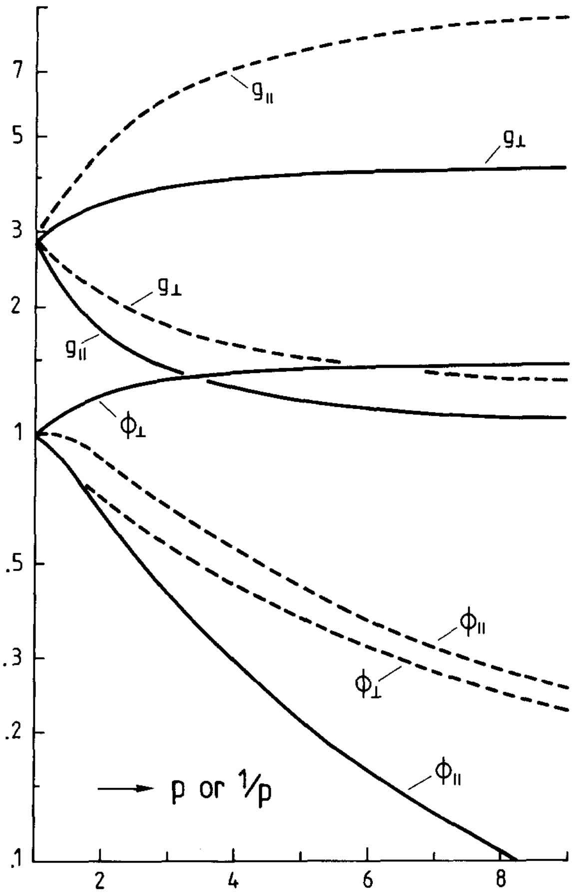

Fig. 5. The function ϕ_j for ellipsoids of revolution ($j = \parallel, \perp$ referring to dipole moments parallel and perpendicular to the symmetry axis) plotted versus the axial ratio p (prolate type, solid curves) or versus $1/p$ (oblate type, dashed curves). In addition the factor g_j of Eqn. 11 is analogously presented for trichotoxin in pure octanol (using $\varepsilon_o = 2.5$, $\varepsilon = 10.5$).

one obtains

$$a = \left((3/4\pi) p^2 \gamma_s (nM_1/N_A)\bar{v}\right)^{1/3}, \quad b = a/p \tag{9}$$

(N_A, Avogadro's number). In particular, for 25°C and octanol being the solvent the relation

$$a = 8.16\left(n\gamma_s p^2\right)^{1/3} \text{Å} \tag{10}$$

is deduced.

Regarding the individual contributions to the total dielectric increment the Debye-Onsager approach [21] implies

$$\Delta\varepsilon_{oj} = g_j \frac{\mu_j^2}{3\varepsilon_v kT} N_A (y_n/n)(C_o/M_1) \tag{11}$$

(ε_v, permittivity of vacuum; μ_j, appropriate component of the dipole moment). The factor g_j is a somewhat complex function of ε (i.e., the static dielectric constant of the solution), ε_o (i.e., the 'optical' dielectric constant of the solute material), the axial ratio, p, and the direction of μ_j (i.e., whether it is parallel or perpendicular to the symmetry axis). Some relevant values are given in Fig. 5.

4.3. Examination of particle properties in pure octanol

We consider the dispersion curve shown in Fig. 1. It can clearly not be fitted by a single Debye function. However, two such terms will do. The best choice of parameters is found to be

$$\Delta\varepsilon_{o1} = 0.63 \cdot \Delta\varepsilon_o = 0.435; \quad f_{o1} = 3.0\ \text{MHz} \tag{12a}$$

$$\Delta\varepsilon_{o2} = 0.37 \cdot \Delta\varepsilon_o = 0.255; \quad f_{o2} = 0.66\ \text{MHz} \tag{12b}$$

We shall first investigate whether or not these can actually be related to only two individual dielectric relaxation steps of the solute. Such steps could for instance reflect the parallel and perpendicular dipole modes of a certain single particle type if we postulate that all other possibly existing particles carry practically no dipole at all. Then Eqn. 8 implies the condition

$$\phi_1(p)/\phi_2(p) = 3.0/0.66 = 4.55$$

This may be satisfied in the case of prolate spheroids ($j = 1, 2$ referring to the perpendicular and parallel modes, respectively) but not for oblate ones as can be ascertained by quantitative inspection of the appropriate ϕ-functions in Fig. 5. The resulting value of p is 3.9. Applying Eqn. 8 to the perpendicular mode alone leads to the relation

$$\phi_1 = 1.4 = 0.27 n\gamma_s$$

Since solvation effects on particle volume leading to $\gamma_s > 2$ are absolutely unreasonable, we would necessarily arrive at $n > 2$. According to the reasoning in section 4.1 such an aggregate must coexist with an appreciable amount of monomer particles. These would carry no dipole because of our special postulate. Hence the dipolar structure had to be generated by a conformational transition which occurs upon aggregation. This, however, would be reflected by a non-linear concentration effect of the circular dichroism spectrum in contrast to the measured behavior mentioned above.

There is still the alternative possibility that only one of the two Debye functions in question arises from a dipole on the monomers. If this would be one of Eqn. 12a then Eqn. 8 yields

$$\phi(p) = 0.27\gamma_s$$

With the ϕ-functions for oblate spheroids unrealistically thin structures are obtained. Assuming a prolate shape, the perpendicular dipolar mode can be eliminated because of $\phi > 1$. The case of a parallel dipolar mode and a γ_s in the range of 1.0 to 1.2 results in $p \approx 4$ and semi-axes $a \approx 21$ Å, $b \approx 5$ Å. Such elongated shape appears to be rather unlikely. A more reasonable (i.e. less elongated) shape implies a higher f_{oj}-value.

The points so far discussed suggest that

(i) polar monomers coexist with one or more types of aggregates which are also polar to a certain extent;

(ii) a dipole moment perpendicular to the symmetry axis of a monomer particle (approximated as a spheroidal body) can be excluded since it should give rise to a Debye function beyond the high-frequency tail of the experimental dispersion curve;

(iii) the dipole moment parallel to the symmetry axis of a monomer is associated with a Debye function which falls in the range of that high-frequency tail.

We have therefore good reasons to assume that for high frequencies (> 5 MHz) the dispersion curve can be largely identified by the Debye function of the parallel dipolar mode of the monomeric particles. This approach yields

$$\Delta\varepsilon_{o1} = 0.20(\pm 0.03), \quad f_{o1} = 6(\pm 1) \text{ MHz} \tag{13}$$

On the basis of the f_{o1}-value the monomer dimensions now turn out as $2a = 29.5(\pm 3.0)$ Å (length, $2b = 12.8(\pm 0.8)$ Å (diameter). They are in very good accordance with those of an alamethicin monomer [19] when simply a reduction of length in proportion to the smaller M_1-value of trichotoxin is taken into account. We turn to the calculation of dipole moments. For 25°C and our C_o of 4 mg/ml Eqn. 11 implies that

$$\mu_j = 84\sqrt{\frac{n\Delta\varepsilon_{oj}}{g_j y_n}}\ \text{D} \quad \text{(Debye units)} \tag{14}$$

Depending on the actual aggregation pattern this leads to different results. Let us first check the possibility of micellation. It leaves us with just the two Debye functions of the micellar aggregate to fit the dispersion curve at frequencies below 5 MHz. This can be satisfactorily done only for $f_{o2} \approx 2$ MHz and $f_{o3} \approx 0.6$ MHz, respectively, corresponding to a prolate ellipsoidal shape with $p \approx 3$ (f_{o3} being associated with the parallel dipolar mode). Because of Eqn. 8 one then finds an aggregation number n around 7–8. On the other hand, Eqn. 14 implies that the dipole moments of the micellar aggregate, μ_m (parallel as well as perpendicular to the symmetry axis), relative to that of the monomeric particles, μ_1, are subject to the relation

$$\mu_m/\mu_1 = n(([n-2]/n)(g_1/g_m)(\Delta\varepsilon_{om}/\Delta\varepsilon_{o1}))^{1/2}$$

where Eqn. 3 has been utilized. From the appropriative curve for the g-values in Fig. 5 we obtain $g_1 = 1.6$. With reasonable values for the $\Delta\varepsilon_{om}$ and g_m it eventually turns out that the total dipole moment of the micellar aggregate becomes definitely larger than $n\mu_1$. Since this cannot be true without a conformational change upon aggregation, such micellation will be disregarded. Thus the formation of two or more smaller aggregates is indicated. Assuming the same aggregation affinity for any individual step in the formation scheme, the isodesmic model introduced above becomes applicable ($y_1 = 0.45$). By means of Eqn. 14 we then obtain

$$\mu_1 \approx 45 \text{ D}$$

It must be emphasized, however, that the mode of aggregate formation may certainly somewhat deviate from an ideal isodesmic case. This could involve an appreciable change of y_1 so that the actual dipole moment μ_1 would be correspondingly different. For instance, let the dimerization constant, K_1, in scheme 4a be smaller than the other K_n ($= K$) by only a factor of 2. Then we have $y_1 = 0.23$ leading to a $\mu_1 \approx 63$ D. A similar situation would arise if a certain amount of nonpolar dimers exists due to an anti-parallel association of monomers.

The apparent uncertainty in y_1 thus makes it at present inopportune to give a definite value for μ_1. Naturally this problem does not affect our conclusions regarding the shape of the particle since these did not involve the question of concentration. Evaluations concerning the shape, size and polarity of the aggregates are greatly complicated because of the larger number of adjustable Debye functions which would be available to fit the dispersion curve. Adopting the isodesmic model we can insert Eqn. 5a in Eqn. 11 and take advantage of $\Delta\varepsilon_{oj}$. This then allows an estimate of an average dipole moment of the aggregates per monomer. It is found to be about 40 D. This result implies an essentially parallel orientation of the monomeric dipoles in the aggregates. In addition, the obvious occurrence of comparatively small f_o-values (< 1 MHz) proves the existence of particles which are at least twice as long as the monomer particles. These points suggest a heat-to-tail aggregation mode as already encountered with alamethicin [19].

4.4. The effect of dioxane

Our data obtained with dioxane/octanol mixtures as the solvent can in principle be treated by an approach analogous to that used for the pure octanol case. Unfortunately, we have so far no information regarding the respective average molecular weights. Furthermore the larger α-parameters indicate a comparatively broad distribution of aggregates with a large number of individual Debye functions. Therefore a more detailed analysis of shape and dipole moments cannot be carried out so far in these systems. Some significant general inferences are nevertheless possible.

Turning to the pure dioxane solvent (where $\eta = 1.26$ cP at 25°C, and $\bar{v}$ is taken to be same as in octanol) Eqn. 7 becomes

$$f_{oj} = \{76.8\ \mathrm{MHz}/(n\gamma_s)\}\phi_j(p) \tag{15}$$

For a monomer this yields a frequency > 40 MHz which is obviously far beyond the observed dispersion range. Therefore the dipolar particles must be fairly big aggregates with appropriate large n. Taking the Cole-Cole f_o of 0.22 MHz as an average of the f_{oj}, Eqn. 15 results in a corresponding mean value of the aggregation number given as

$$\bar{n} = 350\{\bar{\phi}/\gamma_s\}$$

($\bar{\phi}$, appropriate average of the ϕ-function). For spherically shaped particles we have $\bar{\phi} = 1$ so that the aggregates would comprise an average of about 300–350 monomers with an overall diameter of 115 Å. In case of spheroids n would be the smaller the more the shape deviates from that of a sphere. If for instance $p = 5$, an average of only about 70 monomers per aggregate turns out with length ≈ 200 Å, diameter ≈ 40 Å. On the other hand, there is also a possibility that disk-like aggregates are formed by side-wise assembly of monomers. In this case we should expect $\bar{n}$ to be around 140 (with an average p of 1/5).

Average dipole moments may be estimated by means of Eqn. 14. Assuming spherical shape we find approx. 1.3 D per monomer. A larger value of about 3.5 D is found for prolate spheroids of $p = 5$.

Analogous considerations regarding the data for trichotoxin in dioxane/octanol mixtures reveal gradually decreasing size and increasing polarity of the solute particles when the octanol content becomes greater. Provided that the aggregates are spherical we obtain the results in Table I.

TABLE I

MEAN AGGREGATION NUMBER, $\bar{n}$, AND MEAN DIPOLE MOMENT PER MONOMER, $\bar{\mu}/\bar{n}$, IN DIOXANE/OCTANOL MIXTURES ASSUMING SPHERICAL SHAPE OF THE AGGREGATES

Octanol content (% v/v)	$\bar{n}$	$\bar{\mu}/\bar{n}$ (D)
0	350	1.3
12	145	3.8
18	120	3.9
35	52	6.6
41.5	30	8.3
67	16	12.3

5. Summary of conclusions

All the dielectric dispersion curves measured clearly reflect a superposition of at least two different dipolar relaxation modes each described by a Debye-type function of frequency. In order to analyse the underlying structures individually we need additional information on the possible kinds of solute particles. Such is to some extent available in the case of pure octanol as the solvent.

The weight average molecular weight, M_w, determined in octanol can most simply be interpreted in terms of an exclusively dimeric form of the trichotoxin. However, this would necessarily be associated with a distinctly higher frequency range of dielectric dispersion than actually observed. Therefore we conclude that a substantial part of the solute must be in a monomeric form whereas there are also certain aggregates to account for the given M_w-value.

Dipolar relaxation is then to be attributed to aggregates as well as single monomers. Since the smaller particles should exhibit the higher dispersion frequencies we have extrapolated a Debye function for the monomers from the high-frequency tail of the overall curve registered. This allows a calculation of the dimensions of the particle from the mid-point frequency. The equation is based on hydrodynamic theory and takes into account the partial specific volume of the solute. It turns out that the single monomer has an elongated structure of about 30 Å in length and 12.5 Å in diameter carrying a dipole parallel to the long axis.

The same approach can be applied to the remaining part of the dispersion curve which must arise from the aggregates. Assuming that these aggregates are essentially uniform the aggregation number becomes fairly large. Then in turn only rather small amounts of the aggregate can exist because of the low average molecular weight. Accordingly each particle must carry a comparatively high dipole moment in order to account for the observed dielectric increment. We even arrive at the result that the dipole moment of a monomer would increase during the aggregation process. A corresponding conformational change does, however, definitely not occur as demonstrated by our circular dichroism data.

We conclude that the aggregates in octanol are actually quite small, preferably dimers and trimers. They must involve a largely parallel association of monomer dipoles. An antiparallel arrangement would lead to mutual cancellation of dipole moments in the aggregates which is in clear contrast to the substantial dielectric increment measured. Furthermore, aggregation does not simply take place side by side because of the fairly low frequency of dielectric dispersion which definitely points to the presence of particles which are at least twice as long as the monomers. Thus some kind of head-to-tail aggregation is suggested in accordance with the analogous situation encountered in the alamethicin case [19]. Similar solvent dependent end-to-end association effects have also been observed for simple α-helical polypeptides [22,23].

Dioxane as the solvent obviously induces a different secondary structure of the trichotoxin since there is much less α-helix content according to the circular dichroism spectrum [24]. This must be associated with the formation of very large aggregates as reflected in the low frequency range of dielectric dispersion. Since we lack so far additional information on the size and shape of the aggregates only some average aggregation number can be estimated for an assumed shape. An upper bound around 350 was found when taking the particles to be spherical. The mean dipole moments per monomer on these aggregates are considerably smaller than that of the monomer in octanol. This may be due to a decrease of the dipole moment when the secondary structure is changed and an additional cancellation of monomer dipole moments because of antiparallel arrangements in the aggregates. At any rate, the aggregation process is certainly of a different nature than the one in octanol.

Mixing dioxane with octanol apparently leads to a gradually decreasing tendency to form the large aggregates and to an increase of the mean dipole moment. According to circular dichroism [24] also more α-helical structure appears. Possibly hybrid types of aggregates are formed involving two different conformations of the monomer. From the dispersion it can be gathered, however, that an appreciable amount of single monomers will exist only if the octanol is at least about ten times in excess of the dioxane.

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