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# The extraordinary dielectric properties of biological materials and the action of enzymes*

**(metastable states in enzymes/coherent vibrations in biological materials)**

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**ABSTRACT** **From very general theoretical considerations it is concluded that many biomolecules (*i*) should have metastable excited states with very high dipole moment, and (*ii*) should be capable of strongly excited giant dipole vibrations with frequencies near $10^{11}$ Hz. Experimental evidence available so far seems to support these postulates. It is suggested that the two postulates should be of importance for the action of enzymes, and relevant experiments are proposed.**

## 1. Introduction

In terms of the experience accumulated by physicists and chemists in many years of research in the dielectric properties of various materials one is tempted to consider biological materials as basically similar to others, except for their much greater complication. This analogy may be highly misleading. Thus,† e.g., from measurement of the dielectric increment of a dilute solution of a small molecule one can obtain its vacuum dipole moment $\mu_v$, which slightly differs from its internal moment $\mu_i$, in the solution through the action of the latter's reaction field (ref. 1, § 6). This implies minor differences in molecular properties in vacuum and in solution.

Consider now, however, the case of a water-soluble protein. Its conformation is largely dominated by the interaction of its charged groups with the surrounding water and ions, which thus form an integral part of the structure and hence of the measured dipole moment. Connected with this is another feature that might lead to great variation in the measured dipole moment and in some other properties of giant molecules: some biomolecules may possess excited levels with very high dipole moment. Such levels tend to be stabilized (become metastable states) through internal and external deformations and through displacements of counter ions. They thus are a property of a larger structure involving the molecule and its surroundings. A molecule might be lifted into the metastable state through the action of electric fields, e.g., when built into a membrane through the high electric field existing there.

This high electric field of order $10^5$ V/cm maintained in many biological membranes represents quite an extraordinary dielectric property; ordinary materials would suffer dielectric breakdown in such fields unless special care were taken. In biology, if one accepts the principle of evolution on a molecular scale, one may ask which task such a field must fulfill.

A possible answer arises from the difference of electrostatic energy of a unit change across the membrane (about $10^{-6}$ cm), which at room temperature $T$ is about 3 $kT$. It suggests that transport of single charges obtains a significance against thermal noise.

Evidence has also been presented (2) that application of external fields to biological systems usually becomes significant when the field energy available in the system exceeds $kT$.

Biological systems perform tasks that on some occasions are highly sophisticated and border the limit imposed by quantum mechanics. Thus the eye may be compared to an image converter sensitive to a single light quantum. Certain fish are sensitive to electric fields so weak that the electrostatic energy of a single $kT$ requires a volume of the order 1 $cm^3$ [Bullok, T. H. (1974) in *Work Session on Brain Interactions with Weak Electric and Magnetic Fields*, to be published]. Such performances require the use of collective properties of assemblies of biomolecules, and one should expect certain prevalent types of collective behavior such as coherent vibrations, as first proposed in 1967 (3). Evidence for their importance in biological systems has recently been obtained (4, 5).

The extraordinary high catalytic power of enzymes belongs to the biological properties that seem to indicate collective behavior of the whole enzyme molecule. Some time ago it was suggested (6) that enzymes do possess metastable states with high dipole moment and are capable of collective motion. From an analysis of the chemical action of many enzymes, D. E. Green (7) has independently reached similar conclusions.

## 2. General theoretical features

Molecular evolution has led to the development of a number of properties that cannot be predicted from theoretical considerations alone, but require intimate collaboration with experiment. Theory can and must provide, however, the general concepts in terms of which experimental evidence should be discussed; activities in particular cannot be discussed in terms of structure alone, except in the realm of linear response, which is unlikely to hold in the case of biological activity.

High electric fields in membranes ($10^5$ V/cm or more), abundance of fixed and mobile ions (yielding $10^5$ V/cm at a distance of $10^{-6}$ cm from a unit charge), suggests strong electric polarization in many molecules. Such polarization will deform the molecule and lead to a nonlinear response invariably connected with a decrease in electrostatic energy.

When applied to a finite system like a giant molecule, the result of calculation (8, 9) implies the existence of a metastable state with high dipole moment, say, $\mu > \mu_0$ where $\mu_0$ refers to the ground state. In a sufficiently strong external field $F$ such that $(\mu - \mu_0)F$ is larger than the energy difference of the metastable and the ground state, the excited state will be depressed below the ground state, but even smaller fields will lead to increased thermal excitation of the polar state.

The results presented above are based on a coupling of

* Partly based on the 1974 J. B. Whitehead Memorial Lecture.

† See ref. 1 for basic concepts and general theory of dielectrics.

polar with elastic modes of the system, and they result in its deformation when the metastable state is excited. In actual cases such deformations might imply larger conformational changes which cannot be treated in such general fashion. One further general consequence does arise, however, for the change in dipole moment upon excitation will frequently lead to rearrangements of counterions and hence to further stabilization of the metastable state. This state may then be expected to have a very long lifetime.

The high dipole moment of the metastable state implies in general its excitation to be coupled with excitation of homogeneous electric vibrations, i.e., giant dipole oscillations. It has been demonstrated theoretically on a simple model that random supply of energy to various polar modes coupled nonlinearly can lead to a strong (coherent) excitation of a single mode provided the energy supply $S$ exceeds a critical $S_0$, $S > S_0$. Frequencies of the order $10^{11}$ Hz have been suggested (3, 10). An essential feature of this model is its coupling with the rest of the material treated as a heat bath in thermal equilibrium at a temperature $T$ which it attempts to impose on the polar modes. When the energy supply to those modes exceeds the threshold, then the most favorable distribution is the one in which all modes but one are nearly in thermal equilibrium while the one mode becomes highly excited. Far-reaching biological consequences may be expected from such excitation.

If this mode represents the giant dipole vibration, then it must be possible to excite it by electromagnetic radiation of correct frequency provided the wavelength is large compared with the dimensions of the oscillating object. A recently published review of Russian work, if confirmed, seems to support this idea (4, 5). It has been found that irradiation of a great variety of biological objects with coherent millimeter waves in the frequency region of $0.5 \times 10^{11}$ Hz can exert great influence on many biological activities provided the power supply lies above a critical threshold. The biological effects are not temperature effects. They show very sharp frequency resonances that do not occur in the optical constants, which indicates that absorption in very small spacial regions only contributes to the biological activity.

The remarkably sharp resonance has a frequency width of order $2 \times 10^8$ Hz. This could be understood on the assumption that the only broadening of the giant dipole vibration arises from its electric coupling with the thin layers of structured water found attached to biomolecules (11), for it has been shown (12, 13) that the dielectric (Debye type) absorption of this water lies in the region of $10^8$ Hz. Clearly the absence of other frictional processes would present most interesting problems. It would be very desirable, therefore, to repeat the relevant experiments and to make sure that the possibility of interference of the coherent radiation in the instruments has been considered.

### 3. Dielectric increment and dipole moment

A great number of investigations of the dielectric properties of dilute solutions of macromolecules in water (and other solvents) have been performed. Let, in the usual way,

$$\epsilon(\omega) = \epsilon'(\omega) + i\,\epsilon''(\omega) \qquad [3.1]$$

be the complex dielectric constant of the material at frequency $\omega$, and let $\epsilon_0(\omega)$ refer to the solvent alone. Then

$$\Delta(\omega) = \epsilon(\omega) - \epsilon_0(\omega) = \Delta'(\omega) + i\,\Delta''(\omega) \qquad [3.2]$$

is denoted as the dielectric increment. An excellent survey on the present state of these measurements has been presented by S. Takashima and A. Minakato (14).

From the well-known dispersion relations, one finds in particular that the static increment $\Delta(0)$ (which is difficult to measure) can be obtained from the measurements of dielectric loss, $\Delta''(\omega)$ by (§ 2 of ref. 1)

$$\Delta(0) = \frac{2}{\pi}\int_0^\infty \frac{\Delta''(\omega)}{\omega}\,d\omega \qquad [3.3]$$

provided one is in the region of linear response. At this stage particular formulae are usually applied to derive from $\Delta(0)$ a value for the square of the dipole moment of the solute. The peculiarities of biomolecules mentioned in *Section 1* do make it desirable, however, to use in the interpretation of $\Delta(0)$ a first stage that is nearly free of particular assumptions based on Eq. [7.39] of ref. 1.

It would be highly desirable, however, to develop an atomic theory of the ionic plasma in this case, in particular in connection with nonlinear properties which could shed much light on the possible existence of highly polar metastable states discussed in *Section 2*, for an external field of sufficient strength would not only decrease the energy of such a state (if oriented in the field), but by acting on the distribution of counterions could in principle tend to redistribute them in a way which would favor excitation of the polar state.

Recent experiments on DNA do in fact point in this direction. Thus (see § 3 of ref. 14) measurements of the dielectric increment in fields of the order 1 V/cm have led to the conclusion that the DNA molecule does not have a permanent dipole moment, but that counterion motion will account for the high value of $\Delta$. Measurement of electric birefringence, on the other hand, carried out in very much stronger electric fields, lead to the conclusion of a permanent dipole. If confirmed, this would provide an important example for the theoretical postulates of the existence of highly polar metastable states discussed in *Section 2*. Excitation of this polar state would lead to a breaking of an electric symmetry and through the arising internal electric field could have far-reaching biological consequences.

Small proteins, in contrast to the above, do not appear to exhibit great ionic displacements (see § 2 of ref. 14). Results are then frequently discussed in terms of a permanent dipole moment $\mu$. This $\mu$ must then be considered as composed of the rather large dipole moments of the constituent amino acids, of polar bonds, and of the charges on the various constituent groups which do depend on the pH of the solution. Careful measurement of the dipole moment of myoglobin (15) has led to the conclusion that the charges on the individual groups alone can account for the measured value. This would imply exact cancellation of the very considerable intrinsic moments of the individual amino acids.

If this result would hold for other water-soluble proteins as well, then it would suggest a new principle of conformation: charged groups are placed outside (in contact with water) such that their intrinsic dipole moments cancel. Clearly one would expect then the energy of interaction to be a minimum. Calculations to this effect have not yet been performed. They must go beyond nearest neighbor interaction, and in fact require consideration of the interaction of each charged group with each other group. Interaction between dipoles is of a rather complicated nature, but it should be of significance that the magnitude of the interaction energy of the dipole moments of two amino acids (larger than 10 Debye units) at a distance of $10^{-7}$ cm can have a magni-

tude of the order of 5 $kT$ but depends of course on the directions of the dipoles. One might expect then that this type of interaction will also be significant for self assembly of larger units.

While minimizing of the free energy is frequently suggested as the relevant criterion for the particular conformation, another viewpoint suggests the possibility of special pathways (16) along which the protein can more easily fold than along others. In terms of evolution on a molecular scale, one might be tempted to suggest that both principles hold so that out of the enormous number of possible proteins only those for which both principles can be satisfied have been selected.

## 4. Strong field effects

It has been shown in *Section 2* that we may expect biomolecules to possess metastable excited states with very high dipole moment and that those states may be further stabilized by appropriate displacements of counterions. Experiments in high external fields may be expected to yield information about this conjecture. In fact the measurements on birefringence of DNA (14) mentioned in *Section 3* do point to this direction, as do preliminary results (17) on the field dependence of powders and solutions of lysozyme. It should be emphasized again that in this context the actual molecule and its environment of counterions must be treated as a unity.

Encouraging results have already been obtained with the help of the electret method (18), in which the material is subjected to a strong field (order $2 \times 10^4$ V/cm). Subsequently the temperature is lowered drastically. Electrets are materials which after removal of the field retain some of their polarization. As a next step the temperature is very gradually increased and the release of polarization is measured as a function of temperature. Curves with a number of peaks are then obtained which separate contributions due to mechanisms with different activation energies. The authors have investigated in detail the enzyme trypsin in the form of a powder with various degrees of humidity. They found a very large electret effect, indicating very high polarization, with four different peaks both in the dry and in the humid state, indicating four different mechanisms. The one at the lowest temperature, at about −120°, has nearly equal height in both dry and humid states, while in the case of the other three peaks (−50°, −30°, and 40°) the hydrated state presents an amplification of the dry state, i.e., water acts as an amplifier of some of the high field polarization.

In an attempt at interpretation, we notice first of all that elastically bound charges do not contribute to the electret effect. Also, from measurement of the dielectric increment of dilute solutions of a number of enzymes in weak fields (see *Section 3*) we know that their small dipolar groups are not mobile. In strong fields they possibly might be affected, but considering that $\mu F/kT$ ($F$ = applied field, $\mu$ dipole moment of a dipolar group, e.g., 5 Debye units) is only of the order 0.02, we may conclude that these groups do not make an appreciable contribution.

We also know from dielectric measurements in strong fields at room temperature in humid powder (17) that the water's main action probably consists in facilitating the motion of counterions. The three peaks that are amplified in the humid case should thus be due to the motion of ions, whereas the peak at the lowest temperature should be due to excitation of the conjectured polar metastable state.

If we accept that the structured water in the immediate neighborhood of proteins freezes at −70° only (see ref. 11), then the following picture emerges.

On polarization in the strong field apart from displacement of elastically bound charges the metastable state is excited. Simultaneously various mobile or weakly bound ions are displaced. The polarization due to these ion displacements is much larger in the humid than in the dry state. On lowering the temperature and removal of the external field the elastically bound charges are released immediately but the remaining polarization is frozen in. On raising the temperature the metastable state is released near −120°. Various types of ions are released above −70° when bound by structured water, and above 0° when bound by ordinary water.

This model can, of course, be modified, and it is to be hoped that further experiments with various initial fields and with a larger range of humidity will provide the necessary information.

Fields of similar strength ($2 \times 10^4$ V/cm), but in the form of pulses of about 20 μsec duration, have been used (19) to induce large conformational changes in dilute aqueous solutions of biomolecules. Amongst the systems considered are polynucleotides consisting of polyriboadenylate, poly(A), and polyribouridylate, poly(U). Depending on the pH and on temperature, these systems can exist in a variety of conformations, and the arrangement of counterions will be expected to play an essential role in the stabilization of a particular conformation. If the electric pulse can remove some of the counterions, then a change in conformation might be expected provided they do not return before this is effected. Neumann and Katchalsky discuss this possibility, but recent experiments (unpublished observations by A. Palma) with H-D replacements have demonstrated that vibrational properties of the attached layers of water are likely to play an important role. This may indicate that certain highly excited modes of the type discussed in *Section 2* may be relevant for the properties of these systems. Further experiments with these systems might, therefore, lead to an understanding of the connection of high field effects with collectively excited vibrational modes.

## 5. The action of enzymes

From the point of view of physics the enormous efficiency of enzymes as catalysts poses the question of whether this involves certain general physical properties common to most enzymes when they are active. It was suggested, therefore, some time ago (6, 9) that the two theoretical concepts discussed in *Section 2*, excitation of highly polar metastable states and of highly excited vibrational states, should be relevant. Independently (7), from an analysis of a great variety of enzymatic reactions, the same conclusion has been reached by D. E. Green. From the point of view of physics these ideas would suggest certain experiments to be carried out while the enzymes are active. They will be discussed below together with their feasibility.

Basically the two concepts will provide for electrical and dynamical storage and transport of energy, and the high polarization may provide for a lowering of activation energies. The theoretical concepts have been derived for very simple models only. These concepts are, however, of such general nature that they should lend themselves to the modifications required in various specific cases. Physical experiments should, therefore, aim at measuring polarization and vibrational properties during the activity or at influencing the latter by acting on vibrations or on polarization.

The existence of low frequency modes in proteins has been demonstrated experimentally [ref. 20 and Genzel, L., Keilmann, F., Martin, T. P., Winterling, G., Yacobi, Y., Fröhlich, H. & Makinen, M. W. (1975), to be published] with the help of the laser Raman effect. The region of $0.5 \times 10^{11}$ Hz, which according to the discussion at the end of *Section 2* should be of special interest, has, however, not been reached yet. It is of importance to realize in this context that when low frequency vibrations are connected with vibrating giant dipoles, then very considerable softening of these modes may arise when two such systems approach. We may think in particular of an enzyme and a substrate molecule having frequencies $\omega_e$ and $\omega_s$, respectively, when separate. The giant dipoles provide a long-range interaction such that the two coupled molecules must be considered as a single system with two frequencies $\omega_+$ and $\omega_-$ even when fairly widely separated. Assuming the vibrations to be harmonic, then one finds in generalizing a previous result (21)

$$\omega_\pm^2 = \frac{1}{2}(\omega_e^2 + \omega_s^2)[1 \pm (q^2 + Q^2)^{1/2}] \qquad [5.1]$$

where

$$q = \frac{\omega_e^2 - \omega_s^2}{\omega_e^2 + \omega_s^2} \qquad [5.2]$$

$Q$ is essentially the ratio of the interaction energy between the two giant dipoles (at a certain excitation) to their internal potential energies. It thus depends on the distance $R$ between the systems (as $1/R^3$ if the distance is sufficiently large) but not on the displacement of the dipoles since both energies depend on them quadratically. The particular case that the giant dipole oscillations in the two molecules consist in correlated oscillations of, respectively, $z_e$ and $z_s$ elastically bound ions of charge $e$ of equal mass $M$ yields

$$Q = \gamma e^2(z_e z_s)^{1/2}/MR^3(\omega_e^2 + \omega_s^2) \qquad [5.3]$$

where $\gamma$ is a numerical constant.

Clearly [5.1] for $\omega_-^2$ holds only as long as $\omega_-^2 > 0$. It is evident that for large molecules when $z$ is large and $\omega$ is small, $\omega_-^2$ may approach zero at finite distances $R$. In this case, of course, the above assumption of harmonic oscillations will break down. Nonlinear terms will become essential and the general considerations made here must give way to treatment for specific cases. It is of importance, however, to realize that even on the assumption of thermal equilibrium the (Van der Waals type) attractive interaction due to those giant dipole vibrations may become very large as $\omega_-^2$ becomes small even though it is negligible for larger distances when $Q^2 \ll 1$, for the interaction energy $I$ of our coupled oscillators is defined as the difference of the free energy at distance $R$ from its value as $R \to \infty$, i.e., when $Q = 0$. Thus

$$I = kT \log \frac{\omega_+\omega_-}{\omega_e\omega_s} = \frac{1}{2}kT \log \times \left[1 - \frac{1}{4}\left(\frac{\omega_e}{\omega_s} + \frac{\omega_s}{\omega_e}\right)^2 Q^2\right] \qquad [5.4]$$

Clearly, as long as $Q^2 \ll 1$ this yields an attractive interaction ($\alpha\ 1/R^6$ when [5.3] holds) which is small compared with $kT$ and hence negligible. With decreasing distance as $Q^2$ increases, however, the attractive interaction may become very large as the mode $\omega_-$ softens.

At the same time, of course, the amplitude of oscillation increases strongly and nonharmonic terms will become essential. It is at this stage that we may contemplate transition of the enzyme to the conjectured highly polar metastable state. For then the available negative interaction energy $I$ may be sufficient to overcome the energy of excitation of the metastable polar state while the chance for this transition should be most favorable when the amplitude of oscillation has the same magnitude as the displacement characterizing the polar state.

As an example, consider the simple case that $\omega_e = \omega_s = \omega$, $z_e = z_s = z$, and that [5.3] holds. Then the previous equations simplify to

$$\omega_\pm^2 = \omega^2(1 \pm Q) \qquad [5.5]$$

$$I = \frac{1}{2}kT \log (1 - Q^2) \qquad [5.6]$$

with

$$Q = \left(\frac{R_0}{R}\right)^3, \quad R_0^3 = \frac{\gamma e^2 z}{M\omega^2} \qquad [5.7]$$

so that

$$I = -\frac{1}{2}kT\left(\frac{R_0}{R}\right)^6 \quad \text{when } R \gg R_0 \qquad [5.8]$$

but

$$I = \frac{1}{2}kT \log \frac{6(R - R_0)}{R_0} \quad \text{when } R \gtrsim R_0 \qquad [5.9]$$

Correspondingly, the magnitude of the force between the two molecules is

$$f = -\frac{\partial I}{\partial R} = \frac{3kTR_0^6}{R^7} \quad \text{when } R \gg R_0 \qquad [5.10]$$

and

$$f = \frac{1}{2}\frac{kT}{R - R_0} \quad \text{when } R \gtrsim R_0 \qquad [5.11]$$

We also note that for $\gamma = 1$, $\omega = 10^{11}$ Hz, and $M$ the hydrogen mass, one finds $R_0 \simeq 10^{-6}\ (10z)^{1/3}$ cm. Furthermore, the mean amplitude $(a^2)^{1/2}$ of oscillation when $zM\omega^2a^2 \simeq kT$ is $(a^2)^{1/2} \simeq 10^{-6}/z^{1/2}$ cm.

These illustrations are meant to point to some of the consequences arising from low frequency polar modes. It is of importance to realize that experiments on enzymes in crystalline form or in solution will not yield values for $\omega_-$ but rather of $\omega_e$. It would, of course, be desirable to find $\omega_-$; this would require optical measurements in the soft mode region while an enzymatic reaction is maintained.

An easier task may be an experimental proof for the excitation of the highly polar state during enzyme activity. Two experimental possibilities arise: (*i*) measurement of the dielectric increment during enzymatic activity; (*ii*) measurement of the electric signal arising from activation of the highly polar state. The first requires measurement of $\Delta$ of the order $\mu_e^2 n_e/kT$ where $\mu_e$ is the dipole moment of the excited state and $n_e$ is the number per unit volume of excited enzyme molecules. $\mu_e \simeq 10^3$ Debye units ($10^{-18}$ e.s.u.) and $n_e \simeq 10^{16}$ per cm$^3$ would give $\Delta \simeq 1$, which is easily measurable.

Case *ii* requires investigation of the increase of electric noise during enzyme activity‡. Let $L$ be the distance of the two electrodes used for the measurement, and $A$ their area. Then if $t_e$ is the time required to establish the excited dipole moment $\mu_e$, the current arising from it is $\mu_e/t_eL$. The total current is the sum of all these individual currents,

$$J_e = \sum_i \left(\frac{\mu_e}{t_eL}\right)_i \qquad [5.12]$$

where $i$ indicates an individual process. Hence, if there is no

‡ B. K. P. Scaife, personal communication.

correlation between individual processes, then the mean square current is

$$J_e^2 = \sum_i \left(\frac{\mu_e}{t_e L}\right)^2 = \frac{\mu_e^2}{t_e^2 L^2} LA\, n_e \frac{t_e}{t_d} \qquad [5.13]$$

where, as before, $n_e$ is per unit volume the number of excited enzyme molecules, and $t_d$ is the duration of the excitation. Suppose the excitation processes have the shape of a pulse of duration $t_e$; then we expect a frequency distribution of the form $(\sin 2\pi\nu t_e)/\nu t_e$ so that the frequency range $\Delta\nu$ is of the order $1/t_e$.

In the same frequency range the fluctuation current of ions with number density $n_i$, relaxation time $t_i$, and mass $M_i$ satisfies

$$J_i^2 = \frac{e^2 n_i t_i\, \Delta\nu\, kT}{M_i(1 + 4\pi^2\nu^2 t_i^2)} \frac{A}{L} \qquad [5.14]$$

so that considering that $\nu t_i \ll 1$,

$$\frac{J_e^2}{J_i^2} \simeq \frac{M_i(\mu_e/e)^2}{kT\, t_d t_i} \frac{n_e}{n_i} \qquad [5.15]$$

where $\Delta\nu t_e \simeq 1$ has been used. Assuming $M_i = 10^{-23}$ z, $\mu_e = 10^3$ Debye units, $t_i = 10^{-14}$ s, and $t_d = 10^{-4}$ s gives about $10^{-3}\, n_e/n_i$ which would require $n_e/n_i > 1$ to be significant. A very different result would arise, however, if individual enzyme excitations would not be independent of each other. In that case the right-hand side of Eq. [5.13] would have to be replaced by a much larger term and the result following from [5.15] would be correspondingly larger.

Experiments along different lines have shown recently (unpublished observations by N. Kolias and W. R. Melander) that illumination of a dilute solution of chymotrypsin by laser radiation leads to considerable increase in enzymatic activity provided the concentration is below 10 μg/ml. The authors interpret this in terms of activation of the metastable (active) state of the enzyme through Raman scattering. The decrease at higher concentrations would then arise from collisions in which the excited molecule is transferred to the ground state. This experiment, in conjunction with measurement of the dielectric increment, could probably give important information on the existence and dielectric properties of the metastable state.

## 6. Conclusion

In conclusion we find that the experiments mentioned in *Section 2* support the concept of the importance of polar vibrations in the $10^{11}$ Hz region for biological activity (4, 5) while the above (unpublished observations of Kolias and Melander) on enzyme activity in conjunction with the high field experiments discussed in *Section 4* support the concept of the existence of highly polar excited metastable states in enzymes. Conclusive evidence could be obtained from the experiments discussed in *Section 5*.


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