ELECTRIC FIELD EFFECTS IN BIOMOLECULAR SYSTEMS

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The aim of this brief communication is to draw attention to some of the general effects of high electric fields on the behaviour of biologically active molecules and especially to consider their influence in the important case of the helical polypeptide structures.

Over the past twenty years Piekara and his colleagues have established the quantitative aspects of electric field effects for a number of molecular processes in liquid media [1]. The significance of these effects does not appear to be generally appreciated by biomolecular scientists. Two of Piekara's findings are of immediate relevance in a biological context.

Modest electric fields (50 kV cm^{-1}) can appreciably change the equilibrium between conformational isomers, enhancing the concentration of the more polar molecular species. This could well operate to produce both chemically and physically more reactive forms from molecular conformations in which polar groups are often intra-molecularly bonded and so partly shielded from reaction. One simple instance must suffice. Numerous pharmacologically active molecules, from aspirin to aureomycin, have intra-molecularly bonded hydroxyl groups: these hydroxyl groups could be "released", usually by rotation around a $(C-O)$ bond, and made the more readily able to bond to different groups or to other molecules by an electric field favouring the more polar "open" configuration: see Figure 1. In should be remembered that even at $5A$ from a univalent ion, the local field (F) might exceed 10^6 volt cm^{-1}, and that the relevant non-linear dielectric effects (usually measured below 10^5 volt cm^{-1}) are proportional to F^2.

Another of Piekara's findings is the ability of an electric field so to change the energy function in hydrogen bonds (I), that a measureable proportion can be transformed, with a proton jump, to the zwitter-ion form (II). (Fig. 2).

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During the past twenty years this "proton mobility" has been repeatedly invoked in speculations pertaining to hydrogen-bonded systems.
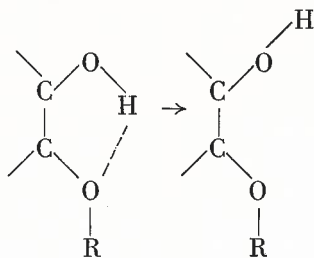

Fig. 1

$$O—H----O \quad (I)$$
$$\downarrow$$
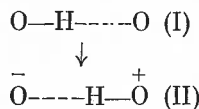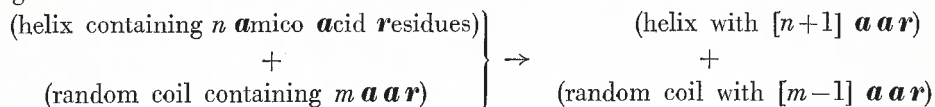$$\overset{-}{O}----H—\overset{+}{O} \quad (II)$$

Fig. 2

Only recently has the spectroscopic [2] and dielectric [3] evidence become rather convincing for its occurrence as an equilibrium process in certain molecular structures. But already many years ago Małecki and Piekara [4] found dielectric behaviour in the alcohols which pointed to proton-jumping occurring within molecular groupings under the action of the high fields (*ca* 50 kV cm^{-1}). Later work [5] on the sterically-hindered alcohols strongly supported Małecki and Piekara's interpretation.

This formation of the ionic structure (II) must often be significant in biological systems. One characteristic feature in such systems is the presence of membranes or cell surfaces at which a preferential adsorption of ions of one charge sign occurs. This will produce very large electric fields near the surface. In such ionic forms as II the molecule may well be able to undergo chemical reaction much more rapidly than as I, or its interaction with the surface may be greatly enhanced by its zwitter-ionic state. Selective penetration through membranes may in some instances be controlled by such changes.

A more specific instance of the electric field effect concerns the kinetics of the formation of the helical configuration in such cases, *e. g.* as poly (γ-benzyl-glutamate) [PBLG] in dioxan solution. This synthetic polypeptide has been much studied as a model of helical molecule behaviour. The molecule is shown by dielectric (dipole and relaxation time) studies to have an essentially linear axis for the helix, closely parallel to which is the direction of the resultant molecular electric moment. [6]. The latter is found (as expected) to be proportional to the helix length (or molecular weight) and, for a molecular weight of 100.000 (degree of polymerization = 685) it is 1540$\pm$50 D. It is well understood that the stability of the helix—which is very unfavourably related to the random-coil form of the molecule in terms of entropy — is a consequence of the energy release on the formation of the continuous series of H-bonds, (N—H---O = C) which hold successive turns of the helix in place. However, this energy term in no way suffices to explain the kinetics of the rapidly reversible random coil $\rightleftharpoons$ helix process.

Recently, Seelig and Schwarz [7] have shown in low-field, variable frequency dielectric studies that the step of one amino-acid residue being transferred from the random-coil to the regular α-helix for the same molecule can be "seen" (as dielectric relaxation theory leaves no doubt should be generally possible) as an absorption feature located near 10^4 Hz.

The rate coefficient thus defined for this step, corresponding to the growth of the helical configuration in the molecule

$$\left.\begin{array}{c}\text{(helix containing } n \text{ } \boldsymbol{a}\text{mico } \boldsymbol{a}\text{cid } \boldsymbol{r}\text{esidues)}\\+\\\text{(random coil containing } m \text{ } \boldsymbol{a}\boldsymbol{a}\boldsymbol{r}\text{)}\end{array}\right\} \rightarrow \begin{array}{c}\text{(helix with } [n+1] \text{ } \boldsymbol{a}\boldsymbol{a}\boldsymbol{r}\text{)}\\+\\\text{(random coil with } [m-1] \text{ } \boldsymbol{a}\boldsymbol{a}\boldsymbol{r}\text{)}\end{array}$$

is found to be as rapid as diffusion of the amino acid residue through a distance of approximately its own length (ca 5A) will allow. This means the unit locks into the correct position on the helix almost as rapidly as molecular motion makes possible. It is at this stage that the great decrease of entropy might well have made the rate of helix formation slow. This entropy factor is often dominant in rates of chemical reaction where specific configurations are needed — e. g. in ring-closure mechanisms.

In the rate of helix formation it may well be that the electric-field factor plays an important role. The electric dipolar field of the helix, constantly directed along the axis, will exert a significant "organizing" influence. At 5A from a dipole of 3.5D, which is the moment along the axis of each ($>$N—H——$-$O$=$C$<$) link in the helix, the field may well exceed 10^5 V cm^{-1}. Once a turn or two of the helix is formed, a large electric field becomes available to attract and orient the dipolar groups of the residue next to be incorporated in the helix. It is the energy term, F(helix) cos θ, where μ_r is the electric dipole associated with the adjacent residue unit and F (helix) is the local field due to the helix already formed, which helps to overcome the unfavourable entropy of activation in the kinetic process of helix formation.

This suggestion would perhaps be regarded as largely speculative were it not that

(*i*) molecules are little other than special patterns of electric charge with the multi--charge positive centres carrying the mass. It is then almost inevitable that structural features and molecular interactions persisting over appreciable distances will involve the presence of long-range electric fields;

(*ii*) the field F (helix) certainly exists and has a highly directional character;

(*iii*) it is certain that some favourable energy term associated with the helix configuration is needed to sustain its growth kinetically, *i. e.* in the rate process, and quite apart from equilibrium considerations. (The equilibrium stability of the helix is determined by the energy made available by the complete hydrogen-bonding system which the helix structure ensures);

(*iv*) such kinetic observations as are available [5] suggest that the helix is formed in some co-operative zip-like process. The strong unidirectional electric field of the helical structure provides, in a simple and effective molecular way, the necessary specific condition for this to occur.

Some further aspects of the helix properties seen in the high field studies must finally be summarized. We have recently shown [8] that thanks to its very large electric moment, it is possible to follow the non-linear response in high fields of the dielectric displacement for quite dilute solutions of the helix: in suitable solutions, up to 90 per cent of "dielectric saturation" is attained at 60 kV cm^{-1}. The behaviour essentially follows the Langevin function with some modifications arising from the non-dipole polarizability of the helix and its anisotropy, as recently expounded by Kielich [9].

The dipole relaxation time of the typical helical solute molecules is less than a milli-second. Accordingly, a pulsed field of longer duration than τ (dipole) suffices to orient the helix and that to different equilibrium extents depending on the voltage amplitude of the approximately square-wave pulse. During the orientation of the helix in this way it is possible also to measure the dielectric properties at 6 MHz by the usual heterodyme beat methods [10]. This has allowed us to do two things (11):

(i) to show that Kielich's representation [12] of the dielectric behaviour of polarizable molecular systems in high fields provides a very good representation of our observations:

(ii) to evaluate the anisotropy of polarizability at 6MHz $(\alpha_{\parallel} - \alpha_{\perp})$ where $\alpha_{\parallel}$ is the 6MHz polarizability along the axis of the helix, $\alpha_{\perp}$ the corresponding value (assumed to refer to a circular cylinder) perpendicular to the axis. Such a measurement is equivalent to determining the Kerr constant for the solute at 6MHz.

Measurements of the Kerr effect for PBLG in the visible (D — line) are already in the literature. For solute of mean molecular weight ca 220.000 (such as we have used) they show $(\alpha_{\parallel} - \alpha_{\perp}) D = 1400 \pm 100 \times 10^{-24}$ cm^3 molecule^{-1}. At 6MHz $(\alpha_{\parallel} - \alpha_{\perp})$ 6MHz $= 5500 \pm 200 \times 10^{-24}$ cm^3 molecule. Of especial significance is the fact that $(\alpha_{\parallel} - \alpha_{\perp})$ is five times as large at 6MHz as at visible frequencies. Few small or medium sized molecules have α (microwave) $> 1.2 \alpha_D$, and the factor of five for the helix implies there is a very unusually large "atomic polarizability" in the helix. This must arise from an abnormally large flexibility in the helical structure giving rise to a pronounced dispersion in α (polarizability) and therefore also in ε (permittivity) or n (refractive index) between 6MHz and 10^{14} Hz. Probably this flexibility and the associated absorption frequencies represent the elastic (concertina-type) modes of vibration in the helix.

These vibrational modes in the halix have not yet been recognized in absorption. The bonding between the terms in the helix is that of a H bond. In such molecules as the formic

acid dimer (H. C $\diagup\!\!\!\diagdown$ $\begin{smallmatrix} O\text{---}\text{--}H\text{---}O \\ O\text{---}H\text{---}\text{--}O \end{smallmatrix}$ C.H) the vibration controlled by the H-bond strength

is near 120 cm^{-1} and it seems probable that in the helix the corresponding vibrations may occur between 10^7 Hz and 10^{12} Hz (30 cm^{-1}). Accordingly, the high field studies have drawn attention to an unusual and apparently unexpected characteristic of the helical molecular structure which has yet to be assessed and its implications in biomolecular behaviour still remains to be considered.

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Note added in proof. Drs P. Maurel and A. H. Price in this Laboratory have now measured a distinct absorption in PBLG solutions. Despite the association of the solute, the absorption is relatively narrow, it is almost temperature independent, and its intensity is ample to account for the $(\alpha_{||} - \alpha_{\perp})$ term at 6 GHz. The frequency of maximum absorption between 2 and 15 GHz is approximately inversely proportional to the molecular weight, as expected for a simple spring. Despite these suggestive indications, caution is needed before concluding that the absorption does arise from a spring-stretching mode.

My other colleagues, Dr T. Krupkowski (Warsaw) and G. Parry-Jones (Bangor) have also established unusually large electric field effects due to the micellar structure in solutions of the biologically important compound lecithin. There are novel time factors both in the appearance of the positive effect and in its subsequent disappearance.