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Water structure-dependent charge transport in proteins

(protons/electrons/charge transfer/dielectric dispersion)

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ABSTRACT **Dielectric and conductivity measurements are reported for bovine serum albumin as a function of hydration. Strong evidence is found for the existence of mobile charges whose short- and long-range hopping motion strongly depends on the physical state of the protein-bound water. These charges are considered to be protons. Insights into the nature of the electrical properties of protein–methylglyoxal complexes are provided, and the possibilities for correlated proton–electron motions are outlined.**

Modern biology is a molecular biology. The rates of most biological reactions are assumed to be limited by the classical concepts of mass action theories applicable to reacting molecules in solution. The main bearers of life are the protein macromolecules, which are often to be found incorporated into membrane structures. This situation would appear to violate the physical basis of classical mass action theories and suggests that the functioning of such structural proteins is controlled at the submolecular level. The reactivity and subtlety that characterizes living systems also indicates that at its most fundamental level the "living state" operates at the submolecular level of nuclei and electrons (1). Such considerations of the reactivity of living systems led one of us to suggest nearly 40 years ago that the functioning of proteins should be understood by considering their submolecular properties. It was envisaged that one manifestation of such submolecular processes would be the ability of proteins to sustain electrical conductivity (2). However, as shown by the pioneering studies of Eley (3, 4), proteins isolated in their pure and dry condition are poor conductors. The basic reason for this is that the valence and conduction levels of extended electronic states of protein structures are separated by such a wide energy gap that at normal temperatures there is a negligible probability for the intrinsic generation of mobile charge carriers. Formation of charge-transfer complexes with electron-accepting molecules makes it possible for the electronic ground states of a protein to become desaturated of electronic charge and lead to a conductivity sustained by positively charged electron "holes." Similarly, a charge-transfer interaction with an electron donor could result in the appearance of mobile electrons in the protein's conduction energy levels. The possibility that proteins can be so converted from insulators into conductors has been demonstrated by Eley and coworkers (5, 6) in conduction studies on complexes of bovine plasma albumin with chloranil or chlorophyll, and our own studies (7–9) indicate the possibility that aldehydes such as methylglyoxal can act as electron acceptors in charge-transfer interactions with proteins. We believe that it is through such charge transfer that structural proteins are imbued with a submolecular reactivity that is essential for their full biological functioning.

Our attempts to fully understand the basic process by which methylglyoxal, when incorporated into a protein's structure, increases the electrical conductivity and dielectric response of that protein is limited by the fact that at present we have no clear comprehension of the electrical properties of *pure* proteins. A recent review (10, pp. 290–306) of the relevant scientific literature indicates that at present even the very nature and polarity of the dominant charge carrier in proteins is not known for certain. For this reason our recent efforts have been directed towards investigating the dielectric and electrical properties of pure proteins. Our basic viewpoint is that in interpreting such investigations proteins should be considered in the same terms as other amorphous and polymeric materials, for which molecular dipole relaxations, ionic mobility, and electronic conduction concepts in extended and localized energy states must all be given proper consideration. Our interest in such studies has been heightened as a result of our recent (unpublished) work with S. Bone and J. A. McLaughlin, which has indicated that the structural proteins extracted from rat hepatoma exhibit a significantly greater dielectric loss and conductivity than do similar proteins extracted from normal rat liver. A clear understanding of the basic cause for this difference should be of relevance to the general study of the cancer problem.

ELECTRICAL STUDIES

Our studies of dry bovine serum albumin (hereafter referred to as albumin), casein, collagen, and lysozyme have revealed that the dielectric response of the dry material is of the form shown in Fig. 1. This dielectric behavior can be separated into two basic components. In the frequency region extending from around 1 Hz up to 33 GHz and possibly beyond, a weak and broad dielectric loss peak exists centered at about 10 MHz. This leads to the observed ac conductivity having the form

$$\sigma(\omega) = A\omega^s, \qquad [1]$$

in which A is a constant, ω is the angular frequency ($\omega = 2\pi f$, f being frequency), and s has a value close to unity. This behavior in proteins has been noted for albumin† and for cytochrome *c* oxidase‡ and is a characteristic response exhibited by a wide range of elemental amorphous materials (11). This effect can be interpreted in terms of the hopping motion of electrons between localized sites, and the broad frequency response results from there being a spread both of the hopping distance between sites and of the energy barriers across which the electrons hop or tunnel (ref. 11, pp. 59–62). The empirical law of Eq. 1 has

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† Pethig, R. (1972) *Preprints of the 3rd International Symposium on the Chemistry of the Organic Solid State*, Glasgow, pp. 95–100.

‡ Eley, D. D., Rooker, S. J., Landon, M. & Mayer, R. J. (1973) *Biochemical Society, Transactions of the 534th Meeting*, Nottingham, Vol. 1, p. 62.

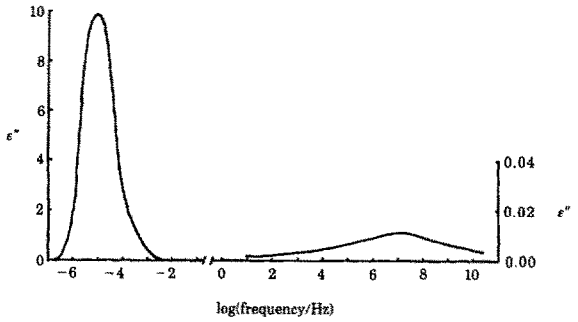

FIG. 1. Frequency variation of the imaginary component of the relative permittivity measured for dry albumin at 294 K.

been explained in terms of a "universal" dielectric response involving the correlated behavior of low-energy excitations in the test materials (12). However, as will be shown below, the weak dielectric response of Fig. 1 can also be interpreted in terms of molecular vibrations of the protein's polypeptide structure.

In Fig. 1 a large dielectric loss peak is seen to occur at very low frequencies, and this has been the most fully characterized for hemoglobin (13) and albumin (14). The relaxation time τ that characterizes this low-frequency dielectric loss follows a temperature-activated law of the form

$$\tau = \tau_o \exp(W/kT), \qquad [2]$$

with the relaxation activation energy W having a value very similar to the activation energy E observed for the steady-state conductivity σ given by

$$\sigma = \sigma_o \exp(-E/kT). \qquad [3]$$

In Eqs. 2 and 3 the factor kT is the Boltzmann energy. This near equivalence of W and E of Eqs. 2 and 3 ($W \approx E = 1.15 \pm 0.05$ eV for the dry protein) suggests that the low-frequency dielectric dispersion and the steady-state conductivity are intimately related. The nature of the loss peak has been found to be independent of the sample thickness and remains in existence when thin sheets of polytetrafluoroethylene or mica are placed between the electrodes and the sample. This indicates that the dielectric dispersion is a true bulk phenomenon. As expected from classical dielectric theory, the existence of the dielectric loss peak is accompanied by a reduction $\Delta\varepsilon$ in the real part of the relative permittivity, with

$$\Delta\varepsilon = \varepsilon_s - \varepsilon_\infty.$$

The magnitude of $\Delta\varepsilon$ can be obtained for the area of the dielectric loss peak by using the relationship

$$\int_{-\infty}^{\infty} \varepsilon'' d(\ln f) = \frac{\pi}{2}(\varepsilon_s - \varepsilon_\infty).$$

For albumin in the dry state the limiting low-frequency value of the relative permittivity is typically of the order $\varepsilon_s = 40$, with $\varepsilon_\infty = 3.1$ being the limiting high-frequency value measured at 100 kHz. From the theories of Debye and Onsager it can be shown (ref. 10, p. 21) that

$$\frac{(\varepsilon_s - \varepsilon_\infty)(2\varepsilon_s + \varepsilon_\infty)}{\varepsilon_s(\varepsilon_\infty + 2)^2} = \frac{N\bar{m}^2}{9\varepsilon_o kT}, \qquad [4]$$

in which ε_o is the permittivity of free space and N is the density of dipolar entities of mean dipole moment $\bar{m}$ giving rise to the dielectric dispersion. From Eq. 4 typically for albumin $N\bar{m}^2 = 9 \times 10^{-31}$ C^2 m^{-1}. Albumin is composed of 575 amino acid residues and has a molecular weight of the order 66,700, so that for our samples of mean specific density 900 kg m^{-3} the above value for $N\bar{m}^2$ corresponds to a mean dipole moment value of 3.3×10^{-28} C m per albumin molecule. The total mean-square dipole moment of a polymer chain composed of polar subunits is given (15) by

$$\bar{m}_t^2 = 3\left\langle P\left\{\sum_1^n \sum_1^n (\bar{m}_n \mathbf{f})(\bar{m}_n \mathbf{f})\right\}\right\rangle_{av}, \qquad [5]$$

in which $\bar{m}_n$ is the vector magnitude of the nth dipole of the chain and $\mathbf{f}$ is a unit vector in the direction of the applied electric field. The sums extend over all of the n dipoles on the chain and the average is to be taken over all of the possible chain configurations and orientations, with P being the probability of occurrence of any particular chain configuration. For a completely random polypeptide chain then it can be shown (ref. 10, p. 50) that to a good approximation Eq. 5 reduces to the form

$$\bar{m}_t^2 \approx 1.1 n \bar{m}_p^2, \qquad [6]$$

in which n is the number of amino acid residues of moment $\bar{m}_p$ in each polypeptide chain. A dipole moment of 3.3×10^{-28} C m for albumin would then correspond to an effective dipole moment of 1.32×10^{-29} C m (4.0 debye units) for each peptide unit. The corresponding result from our measurements on lysozyme of specific density 1100 kg m^{-3} gives an effective moment of 1.23×10^{-29} C m for each peptide unit. Peptide units can be calculated (ref. 10, pp. 44–49) to have a dipole moment of 1.2×10^{-29} C m, so that the low-frequency dielectric dispersions observed for albumin and lysozyme would require complete freedom of rotation for every peptide unit or relaxations of large α-helical regions where the peptide moments would be additive. Such a situation can be considered unlikely for our dry compressed samples. Instead the dielectric dispersion should be considered to arise from the presence of hopping charges within or on the surface of the protein molecules. The effective dipole moment for albumin of 3.3×10^{-28} C m would then correspond to each protein molecule possessing one free charge hopping over a mean distance of 2.1 nm or, for example, to the uncorrelated motions of three charges hopping between sites on average 0.7 nm apart. For the temperature range 292–344 K the product $N\bar{m}^2$ has been found on average to be independent of temperature, whereas (see *Discussion*) gentle chemical treatments of the proteins can significantly alter $N\bar{m}^2$. For reasons that will emerge during this article, we believe that the low-frequency dielectric loss peak of Fig. 1 arises from the hopping motions of protons. For example, support for the conclusion that only one type of charge carrier is involved arises from the narrow nature of the low-frequency dielectric loss peak. This indicates that the charges exhibit a small spread of possible relaxation times, which in turn implies that the charge carrier motion involves a narrow range of hopping distances and potential energy barrier heights. Protons, rather than electronic carriers, are likely to be subjected to such constraints. Other support for the involvement of protons will emerge later in this article.

The weak high-frequency dielectric absorption depicted in Fig. 1 for albumin as accompanied by a reduction of the real part of the relative permittivity of magnitude $\Delta\varepsilon \approx 0.46$ (14). From Eqs. 4 and 6 such a dispersion strength corresponds to an ef-

fective moment of 8.4×10^{-32} C m (2.5×10^{-2} debye unit) per peptide unit and as such could easily correspond to vibrational motions of the polypeptide chain and its polar side groups.

HYDRATION EFFECTS

With increasing protein hydration, the low-frequency dielectric dispersion has been found to progressively shift to higher frequencies (14), with the effective value for $N\bar{m}^2$ remaining unchanged. Defining the characteristic relaxation time τ by $1/2\pi f_m$, in which f_m is the frequency at the maximum of the loss peak, then from the data of ref. 14 and unpublished results the variation of τ with hydration for albumin is as shown in Fig. 2. The relaxation time is seen to depend very strongly on the hydration content and at just under 400 water molecules sorbed per albumin molecule there is a sharp change in this hydration dependence. An extrapolation of τ to a hydration content of around 40 wt %, where completion of the strongly bound "structured" hydration sheaths can be considered to have occurred (16), gives a value for τ of 3×10^{-8} sec. This can favorably be compared with the value of the order 10^{-8} sec estimated by Kirkwood and Shumaker (17) for the dielectric dispersion that would arise from the fluctuations of protons on the surface of an albumin molecule in solution. Our present efforts are directed towards obtaining an experimental value for the limiting high hydration value for τ.

The steady-state conductivity has also been found (14) to be strongly dependent on hydration, and the typical result obtained for albumin is shown in Fig. 3. A change in the conductivity–hydration relationship is seen to occur for a hydration content of the order 9 wt % (approximately 350 sorbed water molecules per albumin molecule). We believe that the sharp transitions observed in the relaxation time and conductivity–hydration relationships are associated with the configuration of the water molecules bound to the protein surface. Support for this conclusion is given by a close analysis of dielectric measurements for hydrated albumin (14, 18), which yields values for the effective dipole moment of the water molecules bound to the protein surface. A detailed account of this analysis will be published separately and we wish here to indicate the main result as shown in Fig. 4. In this figure the effective polarizability *per water molecule* is shown as a function of the total

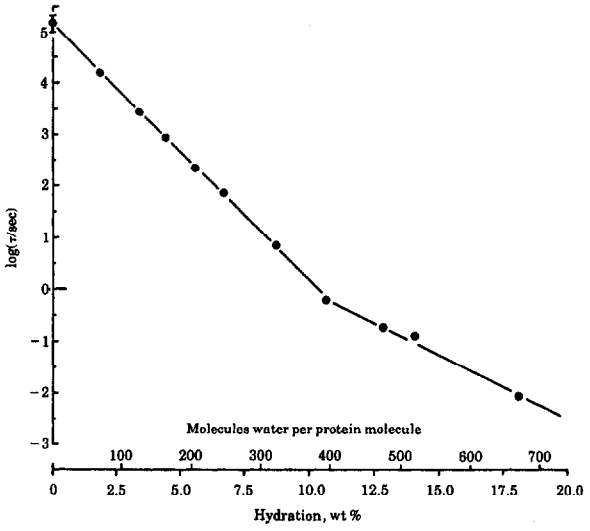

FIG. 2. Variation of the characteristic relaxation time τ of the low-frequency dielectric loss peak for albumin as a function of hydration at 294 K.

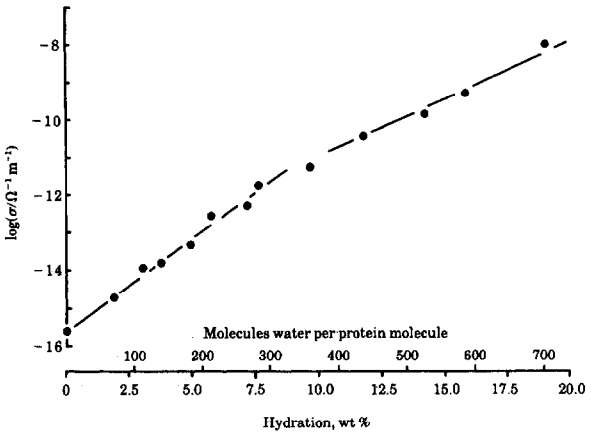

FIG. 3. Variation of the steady-state conductivity σ with hydration for albumin at 294 K.

albumin hydration. The important feature to note is that for hydration contents up to a level corresponding to around 375 water molecules per albumin molecule the polarizability of each bound water molecule remains constant, but thereafter it steadily increases. The polarizability at the lower hydrations corresponds to an effective dipole moment of 2.6×10^{-30} C m (0.79 debye unit), which, because normal bulk water has a moment of 6.14×10^{-30} C m, indicates that the water molecules in the primary sorption sites of albumin are rotationally hindered to a significant extent. Furthermore, the dielectric measurements indicate that water molecules in the secondary sorption sites interact with those in the primary sites so as to loosen their structure and increase their effective polarizability. This is reflected by the changes in the characteristics of Figs. 2 and 3 above hydration values of about 10 wt %. It can be concluded that the dielectric and steady-state conductivity properties of albumin are intimately related to the physical properties of the bound water.

Our previous studies (e.g., ref. 14) have indicated that an empirical relationship of the form

$$\tau \approx \varepsilon_o \varepsilon_\infty / \sigma \qquad [7]$$

exists between the separately measured relaxation time τ and steady-state conductivity σ in any one sample. Recently (19) we have formulated a theoretical basis for a relationship of this

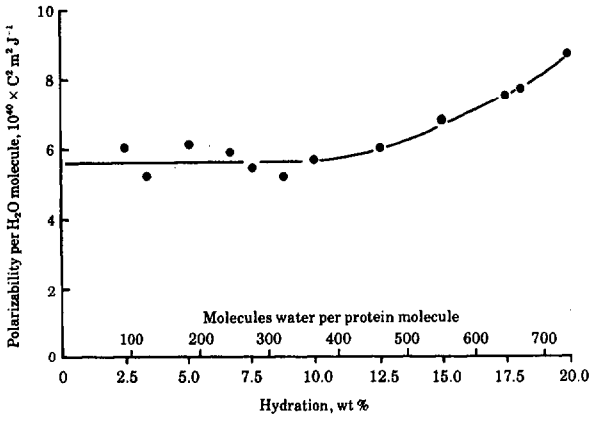

FIG. 4. Variation of the effective polarizability per bound water molecule for albumin as a function of total hydration at 294 K.

form. Basically, generally accepted concepts in conventional dielectric theory and electronic conduction in amorphous solids are brought together to describe the situation in which charges hopping between localized sites can produce both a well-defined dielectric dispersion and steady-state conductivity. Our suggested exact form for Eq. 7 is

$$\tau = \frac{3\varepsilon_o(\varepsilon_s - \varepsilon_\infty)(2\varepsilon_s + \varepsilon_\infty)}{2\varepsilon_s(\varepsilon_\infty + 2)^2\sigma}, \quad [8]$$

and the observed similarity with Eq. 7 has resulted from fortuitous values for ε_s and ε_∞. The relationship of Eq. 8 has been tested satisfactorily for a whole range of protein samples of various hydrations as well as for the perylene–chloranil complex. Those few cases in which the relationship has not agreed with experiment have been understandable in terms of impurities such as sodium and chlorine ions contributing to the total conductivity but not to the dielectric dispersion. For the purposes of this article the significance of the applicability of Eq. 8 is that it indicates that the hopping charges giving rise to the dielectric dispersion are not localized—the charges continue to hop through the whole bulk of the sample to give the observed conductivity. Furthermore, this short-range and long-range hopping transport process is very strongly dependent on the degree of hydration and in particular on the local structure of the bound water molecules.

DISCUSSION

Perhaps the simplest chemical reaction that can occur in a protein is one involving the local transfer of a proton. Also, leaving aside at present the possibility of electronic conduction, the proton, with its radius that is smaller than that of any other ion by a factor of 10^5, should rank as the most mobile charged particle in a protein matrix. Evidence for protonic conduction in proteins includes that for keratin (20), cytochrome *c* and hemoglobin (21), collagen (22), and most recently for single crystals of lysozyme (23). Regarding the proton fluctuation model of Kirkwood and Shumaker (17) and its possible relevance to the low-frequency dispersion of Fig. 1, it is of interest to note that dielectric measurements for myoglobin in solution have indicated that, after macromolecular rotation effects, proton fluctuations contribute the most to the total dielectric behavior (24). Of particular significance is the early work by Baker and Yager (25) on nylon in which they proposed that mobile protons originating from hydrogen bonds contributed to both the conductivity and a low-frequency dielectric dispersion. We believe that such a model is of relevance for our studies described here.

The results of Figs. 2 and 3, together with the theory leading to Eq. 8, can be taken to indicate that the main effect of increasing protein hydration is to increase the effective mobility of the dominant charge carriers. The mobility μ is given by

$$\mu = \frac{q\bar{s}^2}{6kT\tau}, \quad [9]$$

in which q is the charge, $\bar{s}$ is the mean hop distance between sites located on either side of energy barriers of mean height W, and τ is given by Eq. 2. For the case in which the charges escape from a counter charge (equivalent to the "trapping" site being electrically neutral when occupied) the required hop energy over the coulombic potential barrier is given by

$$H = q^2/4\pi\varepsilon_o\varepsilon_r d, \quad [10]$$

in which ε_r is the high-frequency relative permittivity. When $H = kT$ the charge can be considered to be situated at the critical escape distance $d = d_{es}$ from the trapping site. For dry albumin, $\varepsilon_r = 3.1$ to give $d_{es} = 18.3$ nm, and at a hydration of 18 wt % $\varepsilon_r = 6.0$ to give $d_{es} = 9.5$ nm. The hop distance between two such trapping sites for dry albumin would then exceed 36 nm ($2\,d_{es}$) and in Eq. 2 the activation energy W would equal H. Two facts indicate that this model is not fully applicable to our samples. First, a charge q hopping a mean distance $\bar{s}$ will exhibit an effective dipole moment $\bar{m} = q\bar{s}$, so that the measured dipole moment value of 3.3×10^{-28} C m per albumin molecule would correspond to there being no more than one hopping charge carrier for every 17 albumin molecules. A free charge carrier concentration of such magnitude can be considered as being unrealistically small. Second, for such large mean hop distances the charges would experience the full extent of the macroscopic permittivity, which in turn will be directly proportional to $h\alpha$, in which h is the hydration and α is the polarizability variation shown in Fig. 4. From Eq. 2, in which the value for W would be given by Eq. 10, we would then expect Fig. 2 to show τ having a greater rather than decreased hydration dependence for h above 10 wt %. Also because the conductivity σ is given by

$$\sigma = Nq\mu,$$

in which N is the hopping charge carrier concentration and μ is the mobility given by Eq. 9, then in Fig. 3 the rate of change of σ with h should increase rather than decrease as found experimentally. We can conclude, therefore, that the hopping charges do not experience the full macroscopic polarizability and instead hop over relatively short distances (e.g., less than about 1.5 nm), for which the exact conformation of the bound water molecules rather than their collective polarizability is the important factor controlling charge mobility. Such a scheme is compatible with protonic conduction. Such short hopping distances between the "trapping" sites implies that there is an overlapping of neighboring coulombic potential barriers. The diffusion of charges can therefore be envisaged as a process of percolation through "connected" hopping sites, with each hop being coordinated with the movement of neighboring charges so as to avoid unfavorable coulombic, repulsive, interactions. Maximal charge transport will occur when on average the number of occupied trapping sites equals the number of vacant ones (a charge carrier can only hop to an empty site). With J. A. McLaughlin we have chemically modified lysozyme and albumin (e.g., lysine and arginine blocking, dialysis against weak acids and bases) in an attempt to vary the nature of the protein-bound counter-ions. Significant changes in the resulting conductivity and dielectric behavior have been observed to result from such treatments, and they can be taken as evidence for the existence of protonic transport. This work will be published separately.

It should also be noted that repulsive interactions set a limit on the maximal density of electrically uncompensated charge that can exist in a material. This maximal space charge density occurs when the repulsive energy between two like charges, H, is of the order of the available thermal energy, kT. It can be shown by using this criterion in Eq. 10 that for albumin this maximal density of uncompensated charge would correspond to one charge for every 17 protein molecules. From this it may be concluded that uncompensated space charge effects are not of major importance in understanding the conduction phenomena reported here.

Although a strong case for the relevance of protonic conduction has been given above, this does not exclude the possibility that electronic conduction effects can also occur in proteins. In fact, we believe that the coexistence of electron and proton transport may produce a wide range of possible submolecular

electrical activities. For example, recent theoretical studies have concluded that electron transfer can occur across hydrogen bonds and that the rate of such transfer is greatly increased when the electron motions are strongly coupled with those of the protons (26, 27). Also, aromatic amines are capable of the simultaneous production of electron-transfer and proton-transfer molecular complexes (28), and the protonation of peptide side chains can introduce new electronic energy levels in proteins (29). Recent experimental data (30) and theoretical studies (31, 32) indicate that electronic transport in proteins will predominantly occur as a hopping rather than a coherent wave process. This makes the effective mobilities of electrons comparable to those of protons and increases the possibility for some form of correlated activity. So far such effects have not been considered in depth by biological scientists, but the implications to a wide range of phenomena observed in living systems may be extremely important.

Finally, we believe that these studies assist in interpreting the effects observed for the protein–methylglyoxal complexes (7, 8). It now seems likely that rather than introducing a new dielectric loss peak the action of methylglyoxal is to decrease the relaxation time of Eq. 2 by some 3–4 orders of magnitude, so bringing the associated low-frequency dielectric loss peak into a measurable range of frequencies at room temperature. We have found that the protein–methylglyoxal samples take up more water than normal proteins. For low partial pressures of water this may be a major factor. The polarizability of the methylglyoxal molecules linked to the lysine side chains as Schiff bases (33) may also be involved, as well as the proton donor-acceptor nature of the Schiff bases. Such possibilities, as well as the involvement of electronic charge-transfer interactions, should be explored in our efforts to find experimental support for the concept that macromolecules such as proteins provide more the stage than the actors of the drama of life (34).

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