PROTEINS: Structure, Function, and Genetics 28:174–182 (1997)

Electrostatics of Proteins: Description in Terms of Two Dielectric Constants Simultaneously

L.I. Krishtalik,* A.M. Kuznetsov, and E.L. Mertz
A.N. Frumkin Institute of Electrochemistry, Russian Academy of Sciences, Moscow, Russia

ABSTRACT **In the semi-continuum treatment of the energetics of charge formation (or transfer) inside a protein, two components of the energy are inevitably present: the energy of interaction of the ion with the pre-existing intraprotein electric field, and the energy due to polarization of the medium by the newly formed charge. The pre-existing field is set up by charges (partial or full) of the protein atoms fixed in a definite structure. The calculation of this field involves only the electronic polarization (the optical dielectric constant ϵ_o) of the protein because the polarization due to shifts of heavy atoms has already been accounted for by their equilibrium coordinates. At the same time, the aqueous surroundings should be described by the static constant ϵ_{sw}, as the positions of water molecules are not fixed. The formation of a new charge, absent in the equilibrium X-ray structure, results in shifts of electrons and polar atoms, i.e., it involves all kinds of medium polarization described by the static dielectric constant of protein ϵ_s. Thus, in calculations of the total energy, two different dielectric constants of the protein are operative simultaneously. This differs from a widely used algorithm employing one effective dielectric constant for both components of the ion's energy. Proteins: 28:174–182, 1997.**

Key words: proteins as preorganized media; intraprotein electric field; ion charging; ion formation energy; optical and static dielectric constants; reorganization energy

INTRODUCTION

It is widely recognized that electrostatic interactions are of primary importance in the properties and function of proteins (for some recent detailed reviews, see refs. 1–4). From the point of view of electrostatics, proteins are quite specific media. They possess a high concentration of strongly polar groupings (peptide bonds first of all). However, these dipoles are fixed inside a definite structure, and hence their mobility is severely restricted. Therefore, the dielectric response of proteins to an external electric field is rather weak, their static dielectric constant being about 4 (see, e.g., ref. 5). Thus proteins can be defined as *highly polar low-dielectric media*, a combination that is impossible for low-molecular-weight solvents.

In low-molecular-weight liquids, the electric field set up by their dipoles at any point fluctuates around zero. In the presence of a permanent electric field, e.g., upon immersion of an ion into the solvent, a reorganization of the medium takes place, some average permanent orientation of dipoles appears, and their field acquires a non-zero value. On the other hand, in proteins, the permanent component of the average dipoles' field at any point is non-zero, the spatial distribution of the field being determined by the protein structure. The intraprotein electric field exists before introduction of any ion into the macromolecule, and therefore proteins can be defined as "preorganized media" as opposed to the usual solvents.[6] Some fluctuations of the dipoles' field do take place in proteins too, but the amplitude of these fluctuations is relatively low (low dielectric constant).

An ion appearing inside the protein molecule, e.g., at electrolytic dissociation of some side chain, is subject to the action of the permanent electric field existing at the corresponding point of this preorganized medium. This field substantially affects the ion's energy. On the other hand, the ion's own field polarizes the surroundings, resulting in both electronic and atomic polarization (a part of the latter is in a sense an analog of orientational polarization). We will consider here the atomic polarization due to small shifts of atoms and small-angle turns of protein polar groups not accompanied by a major change in the protein conformation. The case of a total restructuring of the macromolecule upon ionization of some group calls for separate consideration, which can hardly be done in the framework of dielectric formalism only. In the case of a substantial shift of only the closest ion's neighbors, a seemingly promising approach combines the molecular level of modeling of the nearest surroundings with the dielectric formalism for the rest of the system (similar to "inner-sphere" and "outer-sphere" reorganization for chemical reactions in solutions). The same is true for

*Correspondence to: L.I. Krishtalik, A.N. Frumkin Institute of Electrochemistry, Russian Academy of Sciences, Leninskii prosp. 31, 177071 Moscow, Russia.
Received 1 April 1996; Accepted 9 September 1996

formation (or a substantial change upon ionization) of covalent bonds between the ion and its ligands; this problem should be treated quantum-chemically.

Thus the energy of ion interaction with a proteinaceous medium consists of two major components: the ion energy in the pre-existing intraprotein field and its charging (Bornian) energy due to the polarization of the medium by the ion. Both these components were considered many times in a continuum dielectric formalism. Such an analysis needs a proper definition of the dielectric constant. The notion of the dielectric constant of proteins in the framework of continuum formalism has been discussed many times.[7–10] The main conclusion of these considerations is that the effective dielectric constant that should be used in calculations depends on the problem, namely, on the approximations used to describe the protein structure. Considering explicitly coordinates and motions of only some part of the constituents of the system, we must describe the behavior of the rest of the particles as an averaged dielectric response, making use of, say, optical, infrared, or static dielectric constant. For example, if the coordinates of all nuclei are assumed to be known (or calculated), and the electronic density distribution is approximately reflected by ascribing to each atom some partial charge, then we can use, in microscopic analysis, atomic polarizabilities or, in a semi-continuum approach, the optical dielectric constant. With the movement of all the nuclei being unspecified, the static dielectric constant is suitable to describe the total response. The different characteristic times of the various modes of polarization should result in different effective ϵs used in the analysis of processes occurring in different time scales, etc.[11,12]

The aim of the present work is to consider how to apply correctly the ideas of refs. 7–10 described above to calculations of the energetics of ion formation. We will show that, in contrast to the protocol usually employed, in calculations of two components of ion energy, viz., the effect of the pre-existing field and the charging energy, two different effective dielectric constants should be used. We start our analysis with a general description of the problem, and we then discuss the technical questions of the application of these results to proteins.

A preliminary account of this study has been reported.[13]

ELECTROSTATICS OF PROTEINS AS STRUCTURED DIELECTRICA

We will consider a protein or other dielectric with a well-defined structure, i.e., with known coordinates of all its atoms. If we knew, in addition, the exact distribution of electron density, we could calculate the electric field at any point of the system considering the charge density distribution in vacuum, i.e., at dielectric constant $\epsilon = 1$. However, such a complete knowledge of the protein's electronic structure is inaccessible, and therefore we must restrict ourselves to an approximate description of the distribution of charge density, e.g., ascribing to each atom (or each bond) a definite partial charge. The values of these charges are usually assumed on the basis of experimental data (e.g., the dipole moments) and theoretical (quantum-chemical) calculations for rather small model molecules, like free amides or short peptides. (We are leaving here aside the problem of the choice of a definite set of partial charges. Different systems of these charges adjusted for different purposes appear in the literature. Most suitable for the problem considered in this paper are the sets evaluated in view of calculations in a semi-continuum approximation. A more detailed discussion of the problem is given in the Appendix.) Now, using these charges, we can, in principle, find the electric field distribution.

However, in these calculations, we should keep in mind that our description of the charge distribution (e.g., partial charges fixed at the centers of atoms) is approximate, and we should take into account the effect of atom charges on the electronic clouds of other atoms, that is, the effect of their electronic polarization. Averaging this effect, we come to a description of the system as a structure formed by fixed charges embedded in a medium with an optical (electronic) dielectric constant ϵ_o. For proteins, this constant can be estimated as about 2.1. (The usual value for aliphatic amides is close to 2.0; here we have introduced some additional correction taking into account that for aromatics, heteroaromatics, and disulfides ϵ_o varies between 2.3 and 2.6.)

It should be noted that the mutual interaction of partial charges of course influences their equilibrium positions, but this effect should not be accounted for as some quasi-orientational polarization because we start with known equilibrium coordinates of an already folded protein.

Let us now consider the formation of an ion inside the protein molecule, for instance, by electron transfer to, or by electrolytic dissociation of, one of the protein groups. On the one hand, this ion appears at a definite place in the structure, and at this point some electric field has existed before formation of the ion. The energy of the newly formed charge is influenced by this field, and the field, as discussed above, can be expressed with the help of the optical dielectric constant ϵ_o. On the other hand, the new ion not only disturbs electronic shells of the atoms of the medium, but also produces some shifts of nuclei, resulting in a new equilibrium structure, but with unknown new atomic coordinates. The situation can be described as a superposition of a set of induced dipoles on the original network of partial charges. That means that the new charge interacts with all kinds of medium polarization, and hence the energy of this interaction depends on the total value of the static dielectric constant ϵ_s.

We can also describe the same situation as an imaginary two-step process. First, the ion is formed at a definite point under the condition that all modes of the protein and water polarization are frozen. In this step, the ion interacts only with the pre-existing field, the latter being determined through the optical dielectric constant of the medium, ϵ_o. Then, in the second step, the full polarization of the protein and its surrounding is released, the electronic density distribution and atomic positions adjust to the field of the newly formed ion, and this results in an ion-medium interaction energy corresponding to the total static polarization ϵ_s.

Our task now is to consider quantitatively the result of superposition of the two effects discussed: the pre-existing field, formed with only the electronic polarization operative; and the static polarization of the medium by the newly formed ion.

We will proceed from the general formula defining the energy of creation of some charge(s):

$$W = \int_V dV \int_{\rho(0)}^{\rho(1)} \varphi(\lambda) d\rho(\lambda) + \int_S dS \int_{\sigma(0)}^{\sigma(1)} \varphi(\lambda) d\sigma(\lambda). \quad (1)$$

Here $\varphi(\lambda)$ is potential; $\rho(\lambda)$ and $\sigma(\lambda)$ are the volume and surface charge densities, all these values being functions of the degree of charging λ. In the course of charging, $\rho(\lambda)$ and $\sigma(\lambda)$ increase from zero to ρ_i and σ_i, and $d\rho(\lambda) = \rho_i d\lambda$ and $d\sigma(\lambda) = \sigma_i d\lambda$. The total field $\mathbf{E}(\lambda)$ can be written as a superposition of the original pre-existing field (the subscript 0) and the field of the newly formed ion, i.e., its own field plus the field of medium polarization produced by this ion (the subscript *i*)

$$-\nabla\varphi(\lambda) = \mathbf{E}(\lambda) = \mathbf{E}_0 + \mathbf{E}_i(\lambda) = -\nabla\varphi_0 - \nabla\varphi(\lambda) \quad (2a)$$

which is equivalent, to within a constant, to

$$\varphi(\lambda) = \varphi_0 + \varphi_i(\lambda) \quad (2b)$$

The indefinite constant in Eq. 2b is omitted, because, in the absence of partial and newly formed charges, we assume both φ_0 and φ_i equal to zero.

In the integration of Eq. 1 with condition (2), we should take into account that φ_0 is the potential of the pre-existing field, and hence it is independent of the parameter of charging degree λ, while $\varphi_i(\lambda)$, the potential due to the new charges, is proportional to λ ($\varphi_i(\lambda) = \lambda\varphi_i$).

$$W = \int_V \varphi_0\rho_i dV + \int_S \varphi_0\sigma_i dS + \frac{1}{2}\int_V \varphi_i\rho_i dV + \frac{1}{2}\int_S \varphi_i\sigma_i dS \quad (3)$$

Using the standard procedure, one can transform Eq. 3 to an equivalent form:

$$W = \frac{1}{4\pi}\int_V \mathbf{E}_0\mathbf{D}_i dV + \frac{1}{8\pi}\int_V \mathbf{E}_i\mathbf{D}_i dV. \quad (4)$$

Here $\mathbf{D}_i = \epsilon_S\mathbf{E}_i$ is the vector of the electric displacement produced by charge density ρ_i. In Eqs. 3 and 4, φ_i, $\mathbf{E}_i$ and $\mathbf{D}_i$ refer to the charge density ρ_i.

In Eq. 3, the first two integrals (or the first term in Eq. 4) represent the energy of new ions in the pre-existing field of partial charges organized in some spatial structure. This expression has the usual form for the electrostatic interaction of a set of charges "*i*" with the potential φ_0, created by charges "0," the value of φ_0 depending on the dielectric constant ϵ_o. The second part of Eqs. 3 or 4 gives the charging energy of new ion(s) in the medium with the static dielectric constant ϵ_s. (This charging energy includes, in the case of formation of two or more ions, their mutual interactions.)

The two components of the free energy called "static" and "relaxation" terms (which, in the present notations, are responsible for the effects of the pre-existing field and of induced medium polarization) were considered in the recent papers by Simonson et al.[14,15] However, they have not analyzed the problem of the dielectric constant for the continuum calculations of the pre-existing field effect.

A more detailed semi-continuum formalism and some problems of its application are described in the Appendix.

ELECTROSTATIC CALCULATIONS FOR PROTEINS

First of all, some refinement of the parameter ϵ_o should be made here. Strictly speaking, in the experimental X-ray structures only the coordinates of heavy atoms are defined, while the coordinates of hydrogens are found from some model construction based on the bond lengths and angles for small molecules. In this sense, the situation here is somewhat similar to our description of electronic clouds. Therefore, some correction for the shift of H atoms from their idealized positions (small changes in the length and orientation of N-H or O-H dipoles, etc.) should be introduced. The effective ϵ_o now becomes not the purely electronic one, but one that includes some part of the infrared polarization. It is difficult to define this part exactly. The correction to ϵ_o we are interested in originates from the stretchings and deformations of the hydrogen's polar bonds having rather high characteristic frequencies. Therefore, it should be close to the effective "quantum" boundary ϵ_q of proteins (the value corresponding to frequencies $\hbar\omega \geq 4$ kT).[16] For water, this effect increases ϵ_q up to 2.1 (compared with $\epsilon_o = 1.8$); for proteins, the total concentration of H atoms is of the same order of

magnitude as in water, but only about 15% of them belong to highly polar N-H or H bonds. Therefore, we estimate for proteins $\epsilon_q \approx 2.2$. On the other hand, if the H atom positions are defined more precisely, only optical value ϵ_o should be used.

We have derived Eqs. 3 and 4 in a general form, and hence they are valid for both homogeneous and heterogeneous systems. Proteins are often surrounded by another medium, in particular water. In this case, the electric fields should be calculated taking due account of the heterogeneity. This means that, in calculation of the pre-existing field, we should use optical ϵ_o for protein but static ϵ_{sw} for water because water is not preorganized. The integrals in these equations are taken over the total volume of the system, including both protein and its aqueous surroundings. In calculations of φ_0 (or corresponding $\mathbf{E}_0$) in this heterogeneous system, we substitute ϵ_o for the proteinaceous part and ϵ_{sw} for the aqueous surroundings. Calculations of φ_i (and/or $\mathbf{E}_i$, $\mathbf{D}_i$) employ ϵ_s and ϵ_{sw}, correspondingly.

In this respect, calculation of the equilibrium energy of ions in a protein globule differs from the calculation of such an essentially non-equilibrium value as the reorganization energy that is relevant to the kinetics of the charge transfer inside the protein. While considering the last problem, one should bear in mind that the pre-existing field, by definition, does not change in the course of reaction, and hence affects only the difference of equilibrium energies of the charge in its initial and final positions. Indeed, the reorganization energy depends on the difference of **D**s (and/or **E**s) in initial and final states, and hence the constant value of $\mathbf{E}_0$ cancels out, only the difference of $\mathbf{D}_i$s ($\mathbf{E}_i$s) being important. The reorganization energy describes the medium repolarization, and so it is connected only with its response to the redistribution of free charges. To calculate this quantity, we have to find the difference of the recharging energies in "slow" and "fast" dielectric media, the "fast" medium being described by optical dielectrics only, for both protein and water, and the "slow" by ϵ_s and ϵ_{sw}.

In calculating the energy of ion(s) in the pre-existing field (first two terms of Eq. 3), we can use the reasonable approximation that ϵ_o in the body of an ion and in its surroundings is practically the same. Then, we can consider φ_0 as a sum of potentials of central forces, i.e., Coulombic potentials set up by each partial charge m. In this case, the energy is equal to the product of the total charge and the potential in the center of this charge:

$$W = \sum_m q_m q_i / \epsilon_o R_m. \qquad (5)$$

In the case of a heterogeneous system, it is often convenient to present the result in the same Coulomb-like form of Eq. 5, adding to the real partial charges q_m their images q'_m (discrete images or their continuous distribution, depending on the system geometry). Each image charge depends, besides geometric parameters, on the ratio of the dielectric constants of the protein and its surroundings (usually, water), e.g., in the case of a planar boundary, on the parameter $K = (\epsilon_o - \epsilon_{sw})/(\epsilon_o + \epsilon_{sw})$. Thus the solution cannot be expressed as a function of a single ϵ_o of protein, but involves some combination of constants.

It should be stressed here that, in considering the pre-existing field, one should include in q_ms not only the partial charges of the neutral multipoles of the protein (peptide groups, etc.) but also the charges of ions that have existed in an ionized form at the pH of the solution used to crystallize the protein (see, e.g., ref. 8). Indeed, "orientational" polarization, i.e., the shift of heavy atoms created by these ions has already been accounted for by the real positions of the protein atoms in a given protein structure. The situation changes when one considers the possibility of neutralization of these ions at some quite different pH. Practically, this is equivalent to the charging of the particle by the charge of an opposite sign. In this case, one should use ϵ_o, as described above, for the effect of the pre-existing field of all other charges, but the whole static dielectric constant ϵ_s in calculation of its charging energy because upon neutralization the pre-existing "orientational" polarization disappears in a normal way.

The last terms of Eq. 3 give the usual Bornian charging energies of each newly formed ion and the energy of their mutual interaction. For the latter, the reasoning similar to that given above leads to Coulomb law ($q_1 q_2/\epsilon_s R$). Of course, the assumption that ϵ inside the ion's body is equal to the surrounding ϵ_s is very rough, and hence, in the case of a complex shape of the ion, one could expect deviation from the simple Coulombic interaction of two point charges. However, if the charge density distribution inside the ion has a spherical symmetry, then the field has the character of the central force field, irrespective of the ratio of the inner and outer dielectric constants. Therefore, the interaction energy retains its simple Coulombic form.

The charging energy of each separate charge (two last integrals in Eq. 3) may be considered, for a heterogeneous system, to be composed of two contributions: the charging in an infinite medium with the properties of the protein, and the interaction of the emerging charge with its own images. The second part can be represented as these integrals with a potential of image charges φ_{im}; in the simple case of a spherical symmetric charge, it equals $\frac{1}{2} \Sigma \varphi_{im} q_i$. The charging energy proper also depends on the character of the charge density distribution in the ion under consideration. In the case of a charge evenly distributed over the surface of a spherical ion of radius a, the energy is $q_i^2/2a\epsilon_s$. (This seems to be a good model for many ions because the excessive charge is concentrated mainly in the outer valence orbital.) As will be described in more detail else-

where, this expression ($q_i^2/2a\epsilon_s$) is valid for the external medium-dependent part of charging energy at any spherically symmetric charge density distribution inside an ion of radius a.

CONCLUDING REMARKS

The mutual interaction of the atomic charges of protein determines the definite equilibrium configuration of the atoms in the folded macromolecule. Therefore, in calculations of the electric field generated by this structure, only the electronic polarization of the medium should be employed because all possible shifts of heavy atoms are already accounted for in their equilibrium coordinates. On the contrary, the formation of a new charge, absent in the equilibrium X-ray structure, results in small shifts of atom positions, revealing itself as an atomic (quasi-orientational) polarization additional to the electronic polarization.

These two cases were discussed, in application to a semi-continuum description of protein electrostatics, in the papers quoted in the introduction. The principal task of the present paper is to apply consistently the notions elaborated in these papers to the calculations of the energetics of charging or recharging of a particle inside the protein. The energy of the process is determined by the sum of two physically different components: the energy of the ion interaction with the pre-existing intraprotein field, and the energy of charging of this newly formed ion in the polarizable medium. Therefore, in semi-continuum calculations of the total energy, two different dielectric constants are operative simultaneously: optical (electronic) ϵ_o for the effect of the intraprotein field and static (ϵ_s) for the charging of the newly formed ion. In the real, heterogeneous systems, one should use ϵ_o of protein and static ϵ_{sw} of aqueous surroundings for computations of the intraprotein field, and ϵ_s and ϵ_{sw} in the charging energy calculations.

Warshel et al.[17] have criticized the widely employed approach by which the static dielectric constant was used in calculating the intraprotein field: thus, the effect of the permanent dipoles was included twice, as a source of the intraprotein field and as a source of dielectric polarization. In the present paper, this contradiction has been eliminated. The field of permanent dipoles is screened dielectrically only by the electronic polarization, and their contribution to the static polarization is taken into account only for the field of external (in respect to these dipoles) charges.

We have shown that, for calculation of the energy of ions in protein, one should employ two dielectric constants of the protein simultaneously, optical and static. This differs from the protocol applied in many works (including our previous ones), in which a single value of ϵ was chosen. (As far as we know, only in our recent paper was the intraprotein field considered as independent of the static dielectric constant, the latter being expressed as evolving in time; however, this question was not treated quantitatively there.[12]) The correct protocol of the semi-continuum analysis of the energetics of charge transfer processes in proteins should make use of different dielectric constants for computation of the two components of the total reaction energy.

This will result in substantial quantitative changes. It is well known that the pre-existing intraprotein electric field brings a marked contribution to reaction energy, usually compensating for a large part of the loss of ion solvation energy (see, e.g., the reviews quoted at the beginning of this paper). With ϵ_o used for the calculation of this field, its effect will increase about 1.5–2 times.

ACKNOWLEDGMENTS

This work was supported by ISF grant MD 9300 and by NIH Fogarty Russian-USA Exchange grant TW00063. E.L.M. was supported by an A.N. Frumkin fellowship.

APPENDIX

In this appendix we give more details on continuum treatment and discuss some aspects of its application.

Introduction of the Effective Dielectric Media

For a description of recharging processes in protein-water medium we formally introduce three effective dielectric media. Generally, the dielectric constants of these effective media inside both water and protein parts of the system may be functions of coordinates (for some related data and considerations see, e.g., refs. 11, 12, 14, 15, and 17–21).

The first effective medium is required to describe the pre-existing field $\mathbf{E}_0$. This field is produced by the pre-existing fixed charges and by the electronic polarization of protein (characterized by the optical dielectric constant, $\epsilon_o(\mathbf{r})$) and by the total polarization of water (characterized by the static dielectric constants, $\epsilon_{sw}(\mathbf{r})$). We may formally introduce the pre-existing dielectric displacement $\mathbf{D}_0$, which is related to the electric field by the effective dielectric constant of the first effective medium, $\epsilon_1(\mathbf{r})$. The source of the fields $\mathbf{E}_0$ and $\mathbf{D}_0$ is the pre-existing charge distribution, $\rho_0(\mathbf{r})$. In the first effective medium the pre-existing charges $\rho_0(\mathbf{r})$ are always at the fixed equilibrium positions. The solution of the electrostatic problem for charge distribution $\rho_0(\mathbf{r})$ in the first medium is given by the potential φ_0. For this effective medium common relations for usual dielectric media are valid.

$$\mathbf{E}_0 = -grad(\varphi_0); \quad div\mathbf{D}_0 = -4\pi \cdot \rho_0(\mathbf{r});$$

$$\mathbf{D}_0 \equiv \epsilon_1(\mathbf{r})\mathbf{E}_0; \qquad \epsilon_1(\mathbf{r}) = \begin{cases} \epsilon_{sw}(\mathbf{r}) \\ \epsilon_o(\mathbf{r}) \end{cases}. \qquad \text{(A1)}$$

As discussed in the main text of this paper, the protein dielectric constant depends on a particular

way of describing protein charge distribution. Therefore, unlike the pre-existing electric field, the pre-existing dielectric displacement depends on the method of description (see Eq. A1). That is why $\mathbf{D}_0$ is a rather formal quantity.

We introduce the second effective dielectric medium to describe the changes in the system upon a slow recharging process. This effective medium is characterized by the effective static dielectric constant,

$$\epsilon_2(\mathbf{r}) = \begin{cases} \epsilon_{sw}(\mathbf{r}) \\ \epsilon_s(\mathbf{r}) \end{cases}.$$

Finally, the third effective dielectric medium describes the changes in the system upon the fast recharging process. This effective medium is characterized by the effective dielectric constant accounting for the inertialess polarization of the medium,

$$\epsilon_3(\mathbf{r}) = \begin{cases} \epsilon_{ow}(\mathbf{r}) \\ \epsilon_o(\mathbf{r}) \end{cases}.$$

For the third medium the dielectric constant of the protein depends on the purpose of the current formalism. For calculation of the total reorganization energy of electron transfer, $\epsilon_3(\mathbf{r})$ is the optical dielectric constant ($\epsilon_o(\mathbf{r}) = 2.1$ and $\epsilon_{ow}(\mathbf{r}) = 1.8$), while for proton transfer $\epsilon_3(\mathbf{r})$ coincides with the "quantum" dielectric constant ($\epsilon_o(\mathbf{r}) \approx 2.2$ and $\epsilon_{ow}(\mathbf{r}) \approx 2.1$). For calculating the activation energy of reaction, keep in mind that only classic polarization modes participate in formation of the activation barrier. Therefore, for both proton and electron transfer the same "quantum" dielectric constant is operative.

Let us consider an explicit change of charge distribution function $\Delta\rho(\mathbf{r})$ proceeding in the second or third effective media. In these cases the common relations are valid

$$\mathbf{E}_i = -grad(\varphi_i);$$
$$div\mathbf{D}_i = -4\pi \cdot \Delta\rho(\mathbf{r});\ \mathbf{D}_i = \epsilon_i(\mathbf{r}) \cdot \mathbf{E}_i. \quad \text{(A2)}$$

Here the subscript $i = 2, 3$ numerates the effective medium.

General Relations for the Electrostatic Work of Recharging Process

Let us assume that the electric field inherent to a medium before a recharging process (the pre-existing electric field), $\mathbf{E}_0$, and the corresponding potential, φ_0, are known. Suppose that the change of the explicit charge distribution function in protein-water system produces a change in the fields $\mathbf{E}_i$ and $\mathbf{D}_i$ (see Eq. A2) so that the fields change from $\mathbf{E}_0$ and $\mathbf{D}_0$ to $\mathbf{E}_0 + \mathbf{E}_i$ and $\mathbf{D}_0 + \mathbf{D}_i$. This is equivalent to the statement that changes in all fields (electric field, dielectric displacement, and polarization) may be described using a continuum approach.

The general expression for the electrostatic work of a recharging process is as follows:

$$W = \int_{\lambda=0}^{1} \left(\int_V \varphi(\lambda) \cdot \frac{d\rho(\lambda)}{d\lambda}\, dV \right) d\lambda. \quad \text{(A3)}$$

Here the integral over λ represents the integral along arbitrary trajectory connecting the initial and final states of the process. Choosing a particular path of the process, we write

$$\mathbf{E}(\lambda) = \mathbf{E}_0 + \lambda \cdot \mathbf{E}_i; \quad \mathbf{D}(\lambda) = \mathbf{D}_0 + \lambda \cdot \mathbf{D}_i. \quad \text{(A4)}$$

From equations $\mathbf{E}(\lambda) \equiv -grad\varphi(\lambda)$, Eq. A4, and relationships between the electric fields and potentials (Eqs. A1 and A2), we derive $-grad\varphi(\lambda) = -grad\varphi_0 - \lambda \cdot grad\varphi_i$. Hence, with an indefinite constant equal to zero,

$$\varphi(\lambda) = \varphi_o + \lambda \cdot \varphi_i. \quad \text{(A5)}$$

Similarly, from equations $div\mathbf{D}(\lambda) \equiv -4\pi \cdot \rho(\lambda)$ and Eq. A4, and relationships between the dielectric displacements and charge distributions (Eqs. A1 and A2), we derive

$$\rho(\lambda) = \rho_0(\mathbf{r}) + \lambda \cdot \Delta\rho(\mathbf{r}). \quad \text{(A6)}$$

Substituting Eqs. A5 and A6 in Eq. A3, we obtain the work

$$W_i = \int_V \varphi_0 \cdot \Delta\rho(\mathbf{r}) dV + \frac{1}{2} \int_V \varphi_i \cdot \Delta\rho(\mathbf{r}) dV. \quad \text{(A7)}$$

We stress here that the relation A7 is valid for an arbitrary dielectric provided that the changes produced by explicit charge redistribution can be described with the aid of a continuum approach using some effective dielectric constant.

Substituting for i the subscript 2 in Eq. A7, we immediately obtain the work of the slow recharging process.

From Eq. A7 we infer two important consequences. First, the energy of interaction of the charge with the pre-existing field is affected directly only by the values of electric field or potential before the process. This means that for calculation of work it is of no importance how the pre-existing electric field or the potential have been calculated. Generally, one may chose any procedures of the calculation of the electric field before the process, e.g., the quantum chemical calculations for the protein dissolved in water or semi-continuum calculations with protein partial charges fixed in the first effective medium.

Second, the work of the recharging process (Eq. A7) is equivalent to the work of charging of effective charge $\Delta\rho(\mathbf{r})$. Thus, the recharging process may be reduced to the charging one with the effective charge.

During a fast recharging process all inertial polarization is frozen. Fast process induces only the change in the electronic polarization. This change in polarization, along with the change in the electric field and dielectric displacement, can be described, in continuum formalism, with the use of the effective charge $\Delta\rho(\mathbf{r})$ in the electronic (third) effective medium. Hence, the work of fast process may be obtained from Eq. A7 by formally substituting the i by the subscript 3.

It may be helpful to have relationships for the potential after slow and fast recharging processes, φ_{slow} and φ_{fast}. During the recharging process the potential is given by Eq. A5. At the final stage of the process $\lambda = 1$, and we get

$$\varphi_{slow} = \varphi_0 + \varphi_2; \quad \varphi_{fast} = \varphi_0 + \varphi_3 \tag{A8}$$

where φ_2 and φ_3 are the potentials obtained from the solution of the electrostatic problems (Eqs. A2) for the effective charge $\Delta\rho(\mathbf{r})$ in the second and third effective media.

Generally speaking, one can operate with any reference state (the state for which the protein structure is available) because the total work of the recharging process depends directly only on the potential before the process under consideration. The procedure of the calculation of, e.g., the charging energy of charge q_2 in the presence of charge q_1 that was absent in the reference equilibrium protein structure (with the potential of the reference structure being φ_0), is as follows. The potential after the charging by q_1 (determined through Eq. A8) gives the potential of the pre-existing field for the second charging process (by q_2): $\varphi_{01} = \varphi_{slow} = \varphi_0 + \varphi_{21}$, with φ_{21} being the potential of charge q_1 in the second effective medium. The required charging energy of charge q_2 is derived through Eq. A7 with effective charge $\Delta\rho(\mathbf{r}) = q_2$ and the potential $\varphi_0 = \varphi_{01}$.

Reorganization Free Energy

To obtain reorganization free energy for the fast recharging process, E_r, we should use the standard definition, namely, reorganization energy is the difference between the energies of the final states obtained at fast and slow charge transfer from the same initial state. This difference may be calculated as a difference in work of the fast and slow recharging process beginning from the same initial reference state.

Thus, for the reorganization energy for redistribution of the explicit charges (with effective charge $\Delta\rho(\mathbf{r})$):

$$E_r = W_3 - W_2 = \frac{1}{2}\int_V \varphi_3 \cdot \Delta\rho(\mathbf{r})dV - \frac{1}{2}\int_V \varphi_2 \cdot \Delta\rho(\mathbf{r})dV. \tag{A9}$$

This equation coincides with that for the reorganization of a dielectric medium derived by Liu and Newton[22] and Marcus.[23] Thus the expression for reorganization free energy for the preorganized protein medium turns out to be the same as for the usual dielectric medium.

According to Eq. A9, the reorganization energy is independent of the potential of the pre-existing field, and one may chose any reference structure, whether it is known or not.

The Relation Between Protein Dielectric Constants and Microscopic Changes of the Protein Structure

An external source of electric field induces protein atomic shifts from the equilibrium reference positions. At the same time, these shifts could be described using the continuum approach of this paper. Therefore it would be possible in principle to get from the experiment the relation between the two dielectric constants of protein and the atomic shifts, if it would be possible to obtain these shifts from structural studies of the protein in the presence and absence of the field source. Here, any source of the external field could be considered, e.g., the oxidized or reduced group of the protein, the external permanent electric field applied to the protein, and so on.

For simplicity, we consider the case of a spherical ion formation in an infinite "uniform" protein that is in equilibrium reference structure. We assume the ion charge distribution function, $q(\mathbf{r})$, to be such that the total charge q is uniformly distributed inside a thin surface layer of an ion of radius a.

The work of such charging is given by Eq. A7 in the second medium, with the effective charge $\Delta\rho(\mathbf{r}) = q(\mathbf{r})$:

$$W = \int_V \varphi_0^r \cdot q(\mathbf{r})dV + \frac{1}{2}\int_V \varphi_2(q) \cdot q(\mathbf{r})dV \tag{A10}$$

where

$$\varphi_0^r = \frac{1}{\epsilon_o}\sum_k \frac{q_k}{|\mathbf{r} - \mathbf{r}_k^r|}$$

is the potential of the pre-existing field at point $\mathbf{r}$ produced by all the pre-existing charges q_k in the equilibrium positions $\mathbf{r}_k^r$ of the reference structure, and

$$\varphi_2(q) = \frac{1}{\epsilon_s}\int \frac{q(\mathbf{r}')}{|\mathbf{r} - \mathbf{r}'|}\, d\mathbf{r}'$$

is the potential created by the charge distribution $q(\mathbf{r})$ in the static medium.

The ion charging results in new equilibrium positions, $\mathbf{r}_k$, of the protein pre-existing charges. Let us discharge the same ion and calculate the discharging

work, W', using the same formula (Eq. A7). Now the sign of effective charge is the opposite, $\Delta\rho(\mathbf{r}) = -q(\mathbf{r})$, and the ion appearing in the new equilibrium structure must be considered a pre-existing charge. We have

$$W' = -\int_V \varphi_0 \cdot q(\mathbf{r})dV + \frac{1}{2}\int_V \varphi_2(-q)\cdot(-q(\mathbf{r}))dV \quad \text{(A11)}$$

with the potential of the pre-existing charges in the new equilibrium structure

$$\varphi_0 = \frac{1}{\epsilon_o}\sum_k \frac{q_k}{|\mathbf{r}-\mathbf{r}_k|} + \frac{1}{\epsilon_o}\int \frac{q(\mathbf{r}')}{|\mathbf{r}-\mathbf{r}'|}\,d\mathbf{r}'$$

and the potential of the charge distribution $-q(\mathbf{r})$ in the static medium $\varphi_2(-q)$.

Then we must demand that the slow recharging process is reversible. This means that the calculated charging and discharging energies must be the same in value but opposite in sign: $W = -W'$.

So, taking into account that $\varphi_2(-q)\cdot(-q(\mathbf{r})) = \varphi_2(q)\cdot q(\mathbf{r})$, we derive from Eqs. A10 and A11

$$\left(\frac{1}{\epsilon_s}-\frac{1}{\epsilon_o}\right)\cdot\frac{q^2}{a} = \frac{1}{\epsilon_o}\sum_k \frac{qq_k}{|\mathbf{R}-\mathbf{r}_k|} - \frac{1}{\epsilon_o}\sum_k \frac{qq_k}{|\mathbf{R}-\mathbf{r}_k^r|}$$

where $\mathbf{R}$ is the radius vector of the center of the ion. This relation can be simplified assuming that the atomic shifts from the reference positions, $\Delta\mathbf{r}_k = \mathbf{r}_k - \mathbf{r}_k^r$, are small:

$$\left(1-\frac{\epsilon_o}{\epsilon_s}\right)\cdot\frac{q}{a} \cong -\sum_k q_k \frac{\Delta\mathbf{r}_k\cdot(\mathbf{R}-\mathbf{r}_k^r)}{|\mathbf{R}-\mathbf{r}_k^r|^3}. \quad \text{(A12)}$$

Here the summation is taken over all pre-existing charges q_k (except the ion q).

The electric field of the ion q placed in vacuum (with charge distribution function introduced above) inside the ion body is absent. Therefore, the formation of such an ion in protein results in shifts of partial charges only outside the ion. Thus the radius a is involved in Eq. A12 as a radius of a protein region where the shifts of partial charges upon the ion formation are absent.

For a general case of finite protein surrounded by water, Eq. A12 is more complicated and includes the dielectric constant of water by the effect of the images of pre-existing charges.

Evaluated from Eq. A12, the relation between the protein dielectric constants may be compared with independent estimates of these constants, and thus the self-consistency of the semi-continuum approach may be studied.

Some Remarks on the Choice of Partial Charges

It is worthwhile discussing the problem of the choice and treatment of partial charges. First we recall that partial charges are not directly observable quantities, and their values depend on the purpose for which they are used. For calculation of the pre-existing field, it is hardly possible to perform calculations of electronic density distribution of the entire protein molecule. Therefore, we must use the data on electronic densities of separate protein fragments. The electric field due to a protein fragment may be approximated by the field of model charge distribution. It is usually convenient to describe this model distribution by a set of point charges at certain positions. For our purpose, the most appropriate set would be a set of point charges that reproduce accurately enough the potential around the separate fragment. Generally speaking, this set is not unique.

It is well known that polarizable surroundings affect the electronic density distribution of solute. Therefore, the values of partial charges approximating the solute charge density are also affected by the surrounding reaction field, which is formed by all kinds of polarization. Thus the most suitable partial charges are those related to the separate fragment placed in dielectric with $\epsilon = \epsilon_s$.

Different sets of partial charges are available in the literature. Along with other parameters, they are designed to reproduce the pair-wise atom-atom interactions and particularly the electrostatic interactions. The latter are screened by the electronic polarization that reduces, in continuum description, the interaction energy by $\epsilon = \epsilon_0$. To avoid the explicit allowance for the screening effect, the partial charges should be scaled in an ideal case, so that the atom-atom interaction of scaled atomic charges will look like the interaction in vacuum, i.e., at $\epsilon = 1$. This scaling procedure can be applied quite rigorously only to the case of charges incorporated in an infinite uniform medium. The application of such sets to the present formalism deserves separate consideration.

Let us consider the Poisson equation for a number of arbitrary disposed charges (both partial and full ones) $\rho_0(\mathbf{r})$ in some dielectrically inhomogeneous medium with constant $\epsilon(\mathbf{r})$:

$$div(\epsilon(\mathbf{r})grad\varphi) = 4\pi\rho_0(\mathbf{r}).$$

We may introduce fictitious charges, permittivity, and potential, $\bar{\rho}_0(\mathbf{r}) \equiv \rho_0(\mathbf{r})/\sqrt{c}$, $\bar{\epsilon}(\mathbf{r}) \equiv \epsilon(\mathbf{r})/c$, and $\bar{\varphi} \equiv \varphi\sqrt{c}$, respectively (where c is an arbitrary positive constant), for which the Poisson equation is also valid

$$div(\bar{\epsilon}(\mathbf{r})grad\bar{\varphi}) = 4\pi\bar{\rho}_0(\mathbf{r}).$$

The electrostatic interaction energy is generally represented by a product of potential owing to some

charge and the value of another charge (more exactly, the spatial integral of this product). Hence, the product of a fictitious charge and a fictitious potential gives the true interaction energy: $\overline{\varphi\rho} = \varphi\rho$.

For the case of the above-mentioned sets of partial charges, the form of the Coulombic interaction between two partial charges in an infinite uniform medium is $\bar{q}_k\bar{q}_j/\mathbf{r}_{kj}$ (here $\mathbf{r}_{kj}$ is the distance between scaled partial charges $\bar{q}_k$ and $\bar{q}_j$), while the true form in continuum representation is $q_kq_j/\epsilon_o\mathbf{r}_{kj}$. Comparing these two forms, we conclude that in our case the scaling factor is $c = \epsilon_0$.

Thus, for proper calculation of the pre-existing field and the energy of the newly formed real charge in this field, we should multiply all values of partial charges adjusted to interactions in vacuum by $\sqrt{\epsilon_0}$. In this case usual values of the dielectric constants for protein and water should be used, e.g.,

$$\epsilon_1(\mathbf{r}) = \begin{cases} \epsilon_{sw} = 80 \\ \epsilon_o = 2.1 \end{cases} .$$

The other way to get correct interaction energy is as follows. We should divide the values of all pre-existing, but partial, charges by $\sqrt{\epsilon_0}$, replace the dielectric constant $\epsilon_1(\mathbf{r})$ by a fictitious one

$$\bar{\epsilon}_1(\mathbf{r}) = \begin{cases} \epsilon_{sw}/\epsilon_0 \\ 1 \end{cases}$$

and then find the fictitious potential of the fictitious pre-existing charges. To find the interaction energy of the newly-formed charge with the pre-existing field, we should also find the fictitious value of the *newly formed* charge (dividing the true value of it by $\sqrt{\epsilon_o}$) and calculate the product of fictitious potential and fictitious newly formed charge. However, in calculating the interaction energy of the newly formed charge with the induced polarization (the second term of Eq. A7 or Eq. 4), the true value of the newly formed charge must be used.

It is worth noting that if one calculates, assuming such a "vacuum" set of partial charges, the pre-existing field for a protein placed in vacuum, the permittivity ϵ_o^{-1} must be assigned to the vacuum, that is, the value that is less than unity! One should be not afraid of such a dielectric constant, because it is only a fictitious quantity required to find a fictitious potential. In this case, the dielectric permittivity of protein is set as equal to 1, and hence, with vacuum fictitious permittivity equal to ϵ_o^{-1}, the image effects on the protein-vacuum boundary should be taken into account.

For the other case, when vacuum permittivity is set to be unity, then such calculations must be considered as performed for protein placed in a uniform medium with dielectric constant ϵ_o.

REFERENCES

1. Harvey, S.C. Treatment of electrostatic effects in macromolecular modeling. Proteins 5:78–92, 1989.
2. Davis, M.E., McCammon, J.A. Electrostatics in biomolecular structure and dynamics. Chem. Rev. 90:509–521, 1990.
3. Sharp, K.A., Honig, B.H. Electrostatic interactions in macromolecules: Theory and applications. Annu. Rev. Biophys. Biophys. Chem. 19:301–332, 1990.
4. Warshel, A., Åqvist, J. Electrostatic energy and macromolecular function. Annu. Rev. Biophys. Biophys. Chem. 20:267–298, 1991.
5. Pethig, R. Dielectric and Electronic Behavior of Biological Materials. New York: John Wiley & Sons, 1979.
6. Krishtalik, L.I. The specific features of enzymes as a polar medium and their role in the mechanism of the enzymatic process. Mol. Biol. (Moscow) 8:91–99, 1974.
7. Gilson, M.K., Rashin, A., Fine, R., Honig, B.H. On the calculation of electrostatic interactions in proteins. J. Mol. Biol. 183:503–513, 1985.
8. Gilson, M.K., Honig, B.H. The dielectric constant of a folded protein. Biopolymers 25:2097–2119, 1986.
9. Honig, B.H., Hubbell, W.L., Flewelling, R.F. Electrostatic interaction in membranes and proteins. Annu. Rev. Biophys. Biophys. Chem. 15:163–194, 1986.
10. Krishtalik, L.I. Dielectric constant in calculations of the electrostatics of biopolymers. J. Theor. Biol. 139:143–154, 1989.
11. Krishtalik, L.I. Fast electron transfers in photosynthetic reaction centre: Effect of the time-evolution of dielectric response. Biochem. Biophys. Acta 1228:58–66, 1995.
12. Krishtalik, L.I. Intramembrane electron transfer: Processes in photosynthetic reaction center. Biochem. Biophys. Acta 1273:139–149, 1996.
13. Krishtalik, L.I., Kuznetsov, A.M., Mertz, E.L. Electrostatics of proteins: Description in terms of two dielectric constants simultaneously. Biophys. J. 70:A225, 1996.
14. Simonson, T., Perahia, D., Brünger, A.T. Microscopic theory of the dielectric properties of proteins. Biophys. J. 59:670–690, 1991.
15. Simonson, T., Perahia, D. Microscopic dielectric properties of cytochrome *c* from molecular dynamics simulations in aqueous solution. J. Am. Chem. Soc. 117:7987–8000, 1995.
16. Kuznetsov, A.M., Ulstrup, J., Vorotyntsev, M.A. Solvent effects in charge transfer processes. In: "The Chemical Physics of Solvation." Part C. Dogonadze, R.R., Kálmán, E., Kornyshev, A.A., Ulstrup, J. (eds.) Amsterdam: Elsevier, 1988:163–273.
17. King, G., Lee, F.S., Warshel, A. Microscopic simulations of macroscopic dielectric constants of solvated proteins. J. Chem. Phys. 95:4366–4377, 1991.
18. Krishtalik, L.I. Conformational mobility of proteins and activation energy of enzymatic charge transfer reactions: An analysis in dielectric formalism. J. Theor. Biol. 116:201–204, 1985.
19. Nakamura, H., Sakamoto, T., Wada, A. A theoretical study of the dielectric constant of protein. Protein Eng. 2:177–183, 1988.
20. Smith, P.E., Brunne, R.M., Mark, A.E., van Gunsteren, W.F. Dielectric properties of trypsin inhibitor and lysozyme calculated from molecular dynamics simulations. J. Phys. Chem. 97:2009–2014, 1993.
21. Simonson, T., Perahia, D. Internal and interfacial dielectric properties of cytochrome *c* from molecular dynamics in aqueous solution. Proc. Natl. Acad. Sci. USA 92:1082–1086, 1995.
22. Liu, Y.-P., Newton, M.D. Reorganization energy for electron transfer at film-modified electrode surfaces: A dielectric continuum model. J. Phys. Chem. 98:7162–7169, 1994.
23. Marcus, R.A. Free energy of nonequilibrium polarization systems. 4. A formalism based on the nonequilibrium dielectric displacement. J. Phys. Chem. 98:7170–7174, 1994.