J Chim Phys (1991) 88, 2701-2708

Dielectric properties of proteins : microscopic and macroscopic theory

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Abstract

A microscopic description of protein dielectric properties [1,2] is directly compared to a simpler, standard, continuum model [4], using cytochrome c as a model system. Relaxation free energies in response to a perturbing point test charge are compared, as well as the reorganization free energy for electron transfer between two cytochromes. The models agree regarding the mean properties of the system, but the continuum model underestimates the spatial variations of the local susceptibility, and greatly overestimates the reorganization free energy for electron transfer.

Nous comparons une description microscopique des propriétés diélectriques des protéines [1,2] à un modèle macroscopique standard [4], prenant le cytochrome c comme systeme modèle. Nous calculons l'énergie libre de relaxation en réponse à une charge perturbatrice ponctuelle, et l'énergie de réorganisation pour le transfert d'un électron entre deux cytochromes. Les modèles concordent quant aux propriétés moyennes du système, mais le modèle macroscopique sous-estime la variation spatiale de la susceptibilité locale, et surestime fortement l'énergie de réorganisation pour le transfert d'électron.

Introduction

The average polarizability within proteins is much lower than within bulk water. By placing the active site in a cleft, removed from bulk water, an enzyme therefore lowers the local polarizability, and reduces the reorganization free energy of any charge

separation steps in the enzymatic reaction. Thus the average dielectric properties of enzymes contribute directly to their function. Simonson *et al.* have suggested, further, that the *spatial variation* of the dielectric properties within proteins may have functional importance [1–3]. For example, the lower the polarizability around an enzyme active site, the lower the activation energy for charge separation. Therefore by providing a polarizability at the active site that is lower than in the rest of the molecule, an enzyme would achieve an additional lowering of its activation energy, beyond what is provided by its average dielectric properites.

To test this hypothesis, we have developed a microscopic description of the dielectric properties of proteins [1,2]. In the macroscopic description, these properties are determined by the dielectric constant ϵ, or the susceptibility $\chi = (\epsilon - 1)/4\pi$, of the protein in response to a uniform field. In a microscopic description, they are determined by the generalized susceptibility tensor of the protein in response to an arbitrary perturbing charge density. In the case of a single perturbing point charge, this susceptibility is a scalar function of the charge's position. Its main components are polarization of the electron cloud (electronic relaxation) and deformation of the 3-dimensional atomic structure (dipolar relaxation). The first contribution can be calculated using an atomic point polarizability model [5]. The second can be calculated using molecular dynamics or Monte Carlo simulations. Simple analytical formulæ can be obtained for both.

The purpose of this note is to compare our microscopic approach to the macroscopic, continuum approach [4], using cytochrome c as a model system.

Methods

Consider a protein and a fixed, perturbing, charge density ρ. The perturbing Hamiltonian V_{tot} contains a "static" term V_{static} and a relaxation term V:

$$V_{tot} = V_{static} + V. \tag{1}$$

The first term is associated with introducing the perturbation while constraining the system to retain its unperturbed structure. The second term is associated with the relaxation after the constraints are removed. The perturbation free energy also has a static and a relaxation component:

$$A_{tot} = A_{static} + A. \tag{2}$$

If ρ is small, V is proportional to the perturbing field:

$$V = -\underline{x} \cdot \underline{f}. \tag{3}$$

$\underline{f}$ denotes the $3n$-vector:

$$\underline{f} = (\underline{f}_1, \underline{f}_2, \cdots, \underline{f}_n), \tag{4}$$

where $\underline{f}_i$ is the perturbing field on atom i, and n is the number of atoms in the system. $\underline{x}$ is also a $3n$-vector and represents the structural relaxation of the system. The relaxation can be characterized by a generalized susceptibility $\underline{\underline{\alpha}}$:

$$\langle \underline{x} \rangle = \underline{\underline{\alpha}}\,\underline{f}. \tag{5}$$

The brackets represent an ensemble average. We showed previously that

$$A = -\frac{1}{2}\underline{f} \cdot (\underline{\underline{\alpha}}\,\underline{f}), \tag{6}$$

and that if the atomic fluctuations are small compared to the distance from ρ,

$$\underline{\underline{\alpha}} \approx \frac{1}{kT}\underline{\underline{M}}; \tag{7}$$

$\underline{\underline{M}}$ is the mean atomic dipole-dipole correlation matrix. The susceptibility is thus determined by the fluctuations of the microscopic dipoles of the unperturbed system.

We define the *scalar susceptibility* α by:

$$A = -\frac{1}{2}\alpha \underline{f}^2. \tag{8}$$

α is a projection of $\underline{\underline{\alpha}}$ onto a one-dimensional subspace; this projection measures the relaxation free energy. For a single perturbing point charge, α is a function only of that charge's position.

The formalism is applied to cytochrome c *in vacuo*. A point test charge is placed successively on each $C\alpha$, and the protein's scalar susceptibility calculated. Microscopic and macroscopic calculations are compared. In the microscopic approach, the electronic contribution is calculated using the atomic point polarizability model [1] and the dipolar contribution is calculated using molecular dynamics, as described previously [2]. In the macroscopic approach, the protein is treated as a continuum characterized by a single dielectric constant ϵ [4]. To account for its self-energy, the test charge is assumed to be a sphere of radius 1.9 Å. Permanent charges are located on ionized side chains and the heme. *Ab initio* values [6] are used for the side chains, and semi-empirical values for the heme. Details will be presented elsewhere. The program Delphi was used to solve Poisson's equation [7].

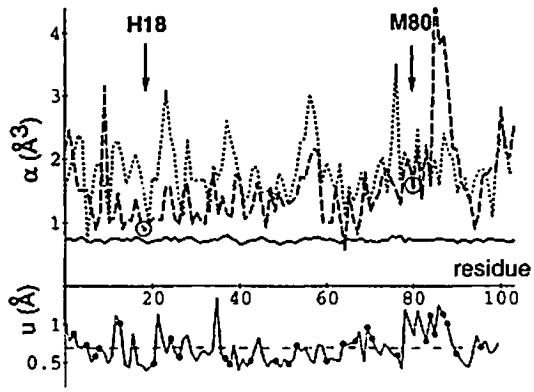

Figure 1: Scalar susceptibility at $C\alpha$ (upper panel) and r.m.s. mobility of residue (lower panel) vs. residue number. Upper panel: dotted curve, α_{mac}; dashed curve, α_{dip}; solid curve, α_{elec}. The black dots on the lower plot indicate charged residues. The axial ligands of the iron, H18 and M80, are indicated by arrows, and circles on the dashed curve.

Results

A test charge is placed successively on each $C\alpha$ and the scalar susceptibility calculated using the macroscopic and microscopic models. Fig. 1 shows the result vs. residue number. The microscopic result is broken down into its electronic (α_{elec}) and dipolar (α_{dip}) parts (only approximately additive [1]). The macroscopic result (α_{mac}) assumes ϵ=2. Fig. 1 also shows the atomic mobility, from a 90 ps molecular dynamics simulation. In Fig. 2 the results are averaged over spherical shells centered at the protein centroid. Table 1 gives the susceptibilities and energies averaged over all the $C\alpha$ positions.

Table 1: Summary of cytochrome c results: scalar susceptibility and relaxation free energy averaged over all $C\alpha$'s. The standard deviations, in parentheses, are spatial deviations, not statistical errors. The average absolute magnitude of A_{static} is given.

Mean susceptibilities (Å^3) and energies (kcal/mol)			
A_{elec}	-23.5 (4.0)	α_{elec}	0.73 (0.03)
A_{dip}	-38.7 (15.3)	α_{dip}	1.57 (0.66)
$A_{mac}(\epsilon = 2)$	-44.4 (6.2)	$\alpha_{mac}(\epsilon = 2)$	1.82 (0.45)
$\mid A_{static} \mid$	18.4 (16.9)		

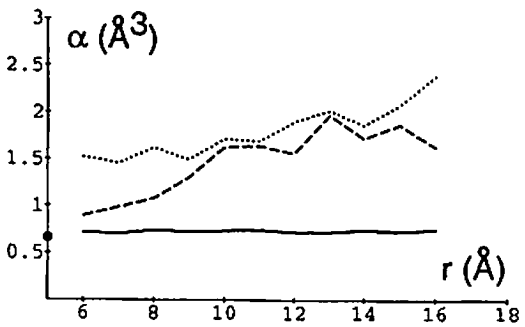

Figure 2: Scalar susceptibility at $C\alpha$, as a function of the $C\alpha$'s distance from the protein centroid, averaged over 1 Å sherical shells. Dotted curve, α_{mac}; dashed curve, α_{dip}; solid curve, α_{elec}. The black dot on the y axis represents the susceptibility at the iron atom (5 Å from the centroid).

We next consider oxidation of the heme. The departing redox electron, which is delocalized over the heme, is viewed as a set of perturbing charges. The relaxation free energy in response to the removal of these charges, A^{oz}, is calculated. 2 | A^{oz} | represents the reorganization free energy for electron transfer between ferro- and ferri-cytochrome c. According to the macroscopic Marcus theory of electron transfer [8,9], this is equal to 4 times the activation free energy for the electron transfer. | A^{oz} | /2 is thus the activation free energy for electron transfer between two cytochrome c's. Table 2 gives A^{oz} calculated from several different models. These are (a) the macroscopic model; (b) the microscopic molecular dynamics model; (c) the microscopic molecular dynamics model corrected for dielectric saturation, as described in ref. 2; (d) the microscopic model using a previous normal mode calculation [1]; (e) a different microscopic model proposed by Warshel & coworkers [8]. This last model assumes that the structural relaxation upon oxidation is equal to the known shift between the crystal structures of ferro- and ferri-cytochrome c; furthermore the relaxation free energy is approximated by the relaxation energy. All five models use the same semi-empirical charge distribution for the redox electron [8].

Discussion

This note compares microscopic and macroscopic theories of the dielectric properties of cytochrome c. We have performed simulations *in vacuo*, thus investigating only the dielectric relaxation of the protein. The important solvent relaxation remains to be analyzed, as well as quantum effects. Our simulations lead to several conclusions.

Table 2: Relaxation free energy for heme oxidation from different models. Harmonic (A^{ox}_{harm}), molecular dynamics (A^{ox}_{dip}), and macroscopic ($A^{ox}_{mac}(\epsilon = 2)$) results. $^*A^{ox}_{dip}$ is the molecular dynamics result corrected for the effect of dielectric saturation [2]. The result of reference 8 is also given.

Relaxation free energies for heme oxidation (kcal/mol)	
refer. 8	-1.7
A^{ox}_{harm}	-1.8
A^{ox}_{dip}	-11.4
$^*A^{ox}_{dip}$	-7.5
$A^{ox}_{mac}(\epsilon = 2)$	-33.5

The macroscopic model agrees approximately with the microscopic model in terms of the magnitude of the susceptibility. The value ϵ=2 is too small to reproduce the total susceptibility $\alpha_{elec} + \alpha_{dip}$, and too large to reproduce either one separately. The variation of α_{mac} along the $C\alpha$ chain is quite different from that of either α_{elec}, α_{dip}, or $\alpha_{elec} + \alpha_{dip}$.

The radial variation of α_{mac} (Fig. 2) is smaller than that of α_{dip}: α_{mac} in the heme region (1.52 Å^3) is almost as large as its mean value of 1.82 Å^3. α_{dip} shows a pronounced minimum in the heme region with a value of 0.63 Å^3 in response to a charge on the iron. This is consistent with the low mobility and low polarity of this region. The uniform dielectric constant of the continuum model, on the other hand, is inappropriate here.

The macroscopic model gives a very large relaxation energy for heme oxidation, which appears to be unrealistic. Note that the result coincides almost exactly with the free energy for embedding a 1.9 Å charge in a 10 Å sphere with ϵ=2, -35 kcal/mol. 10 Å is roughly the depth of the iron beneath the protein surface. The macroscopic result is obtained by calculating a small difference between two large 'solvation' energies, *i.e.* the energy of (a) the electron 'solvated' by the protein, surrounded by a vacuum, and (b) the electron in a homogeneous medium of dielectric constant ϵ=2. This appears to be rather imprecise; details will be published elsewhere. Furthermore, in the macroscopic model, the delocalization of the redox electron is taken into account rather crudely. Here, as in the microscopic models, the redox electron is represented by a set of partial charges $-q_i$, located on the heme atoms. Removal of the electron means inserting the set of perturbing charges $+q_i$. In the macroscopic model, this leads to induced charge (a) located on each partial

charge and (b) smeared over the protein surface. The induced charge located on each perturbing charge is $q_i(1/\epsilon - 1)$. This value does not depend on the presence of the other neighboring perturbing charges. In other words, in the macroscopic model the different partial charges representing the electron do not have to compete for solvation by the protein. The protein can solvate them all at once, because it draws not on a limited number of real, hydrogen-bonding or polar groups in the vicinity of the electron, but on a hypothetical, unlimited, continuum of polarizability.

The macroscopic model also neglects dielectric saturation, a problem that is distinct from, but related to, the previous one. Our work indicates that saturation is important around net charges in proteins [2]. Here, the magnitude of A^{or}_{dip} is reduced significantly by dielectric saturation, from 11.4 kcal/mol to 7.5 kcal/mol. Saturation is estimated by comparing the results of equations 6-8, valid for a vanishing perturbation, with a traditional perturbation free energy calculation, valid for a finite perturbation. This latter approach extracts the perturbation free energy from the ensemble average of $exp(-V_{tot}/kT)$ [2]. For good accuracy, the perturbing charge should be introduced in several steps, as in the original adiabatic charging method of Born [10]. As a first approximation, we introduce it in a single step. It can be shown that this leads to an *underestimate* of saturation. This is one reason why our $^{*}A^{or}_{dip}$ differs from the result of Warshel *et al.* [8], which implicitly includes the effect of saturation.

The normal mode calculation underestimates the atomic fluctuations, on the one hand, and neglects dielectric saturation on the other hand. Because the fluctuations are underestimated, the model leads to a smaller relaxation than the molecular dynamics model, and A^{or}_{harm} is smaller (in magnitude) than A^{or}_{dip}. Neglect of saturation leads to the opposite effect, overestimation of relaxation.

It is difficult to estimate precisely the errors in each of these models, without performing a full-scale free-energy perturbation calculation. However the macroscopic model appears unrealistic, and the molecular dynamics model clearly needs to be corrected for saturation. Therefore our best estimate of A^{or} at present is somewhere between -7.5 and -1.7 kcal/mol.

The low heme susceptibility and relaxation free energy obtained in the microscopic model(s) correlate directly with the function of cytochrome c. This is consistent with our initial hypothesis, the functional role of the spatial variation of the dielectric properties. For a real cytochrome in solution, the solvent makes the predominant contribution to the reorganization free energy for electron transfer. However the relaxation of the protein matrix is not negligible, and therefore the effects seen here could be functionally significant. We find that protein dipolar relaxation contributes just 1-4 kcal/mol to the activation free energy for electron transfer between two cytochrome c's. For hemes in solution, in the absence of protein, this

energy would be much greater.

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