Biophysics, Vol. 46, No. 4, 2001, pp. 558–563. Translated from Biofizika, Vol. 46, No. 4, 2001, pp. 589–594.

MOLECULAR BIOPHYSICS

Influence of the Peptide Environment of Fe_4S_4 Clusters in Proteins on the Energy of Redox Reactions: A Molecular Dynamics Study

A. G. Ivaikina[1], N. K. Balabaev[1], and K. V. Shaitan[2]

[1]*Institute of Mathematical Problems in Biology, Russian Academy of Sciences, Pushchino, Moscow Region, 142292 Russia*
[2]*Moscow State University, Moscow, 119899 Russia*

Received February 5, 2001

Abstract—Methods of molecular dynamics were employed to assess the influence of the peptide matrix and conformational relaxation on the heat effects of redox reactions in two iron–sulfur proteins with markedly different redox potentials. The depth of embedment of the Fe_4S_4 cluster in the protein globule was shown to affect the parameters of chemical equilibrium. The peptide matrix was found to make the definitive contribution in compensating for the energy of Coulomb repulsion in the iron–sulfur clusters.

Key words: iron–sulfur proteins, HiPIP, ferredoxin, redox potentials, peptide matrix

INTRODUCTION

Numerical molecular dynamics (MD) experiments are currently a popular tool in analysis of the structural and dynamic features of biological macromolecules and their complexes. In the chemical aspect, the functioning of the living organism is based on redox reactions. A central part therein is played by the electron transfer chain proteins. Understanding how the organic milieu controls the redox processes is of fundamental importance [1–4]. A large body of experimental material has been accumulated in this field. As simplest objects for a theoretical study of the processes associated with the electronic state of the redox center, we chose two relatively small proteins containing Fe_4S_4 as a prosthetic group: high-potential iron–sulfur protein (HiPIP) and ferredoxin (Fd). These proteins belong to an important class of electron transfer chain proteins bearing iron–sulfur clusters, in which the iron atoms are chemically bonded to the sulfur atoms of cysteine residues. The two representatives have identical Fe_4S_4 clusters but differ markedly in their redox potentials; thus HiPIP represents the group of proteins engaged in redox reactions at potentials from +50 to +450 mV, while Fd represents the low-potential group, –650 to –300 mV.

Ferredoxins are known to play an important role in metabolic processes and nitrogen fixation; they are widespread in living organisms from simple bacteria to higher plants and animals [5]. Bacterial ferredoxins show quite a high extent of homology (especially in the region of prosthetic group binding), indicating a certain evolutionary pathway of these proteins [6, 8]. HiPIPs are also broadly encountered, but their metabolic role in unclear, they have low homology but display very similar spectral properties, which suggests similarity of structure in the polypeptide chain folding around the iron–sulfur cluster [9–11].

In ferredoxins, electron transfer involves the change in the cluster charge state $[Fe_4S_4]^{2+}/[Fe_4S_4]^{1+}$; in HiPIPs, it is $[Fe_4S_4]^{3+}/[Fe_4S_4]^{2+}$. Both proteins in the $[Fe_4S_4]^{2+}$ state (sum cluster charge $-2e$) exhibit analogous optical properties characteristic of a diamagnetic protein; in the other electronic states (sum cluster charge $-3e$ and $-1e$) they show paramagnetic properties associated with an unpaired electron [11–14].

EXPERIMENTAL

The study was carried out on HiPIP of *Rhodocyclus tenuis* (polypeptide chain of 62 amino acid

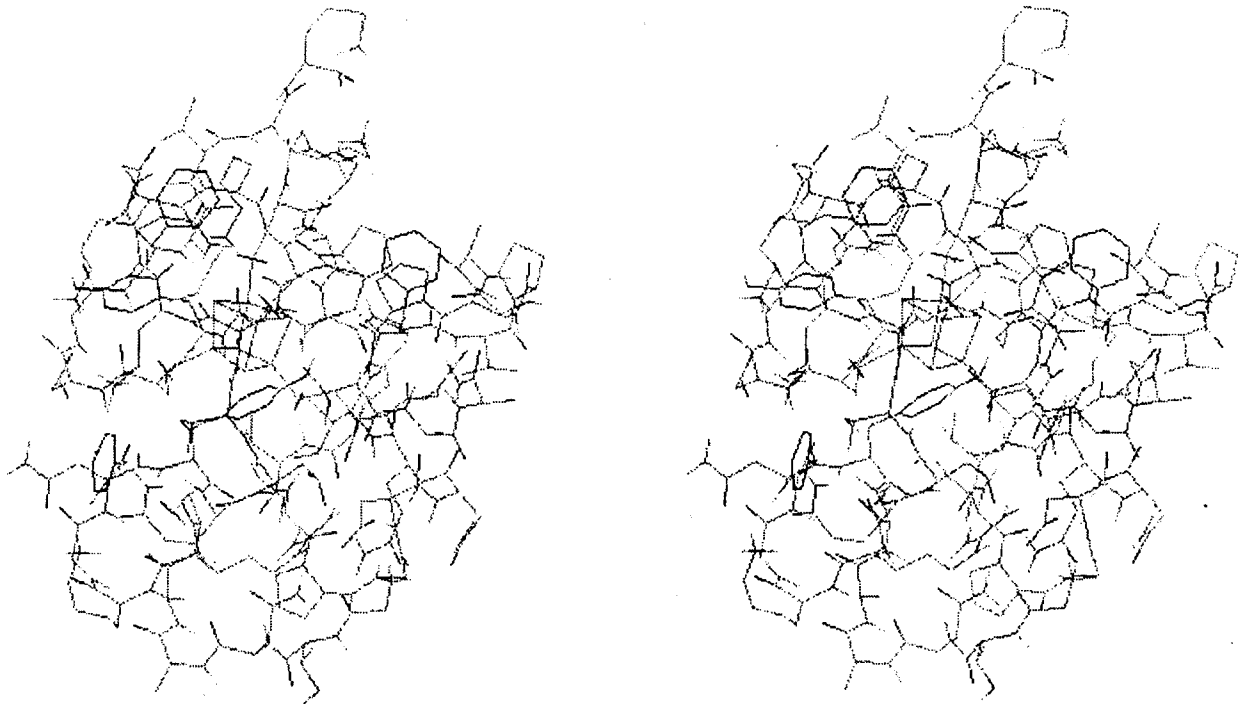

Fig. 1. Structure of the {*Rhodocyclus tenui* HiPIP} protein.

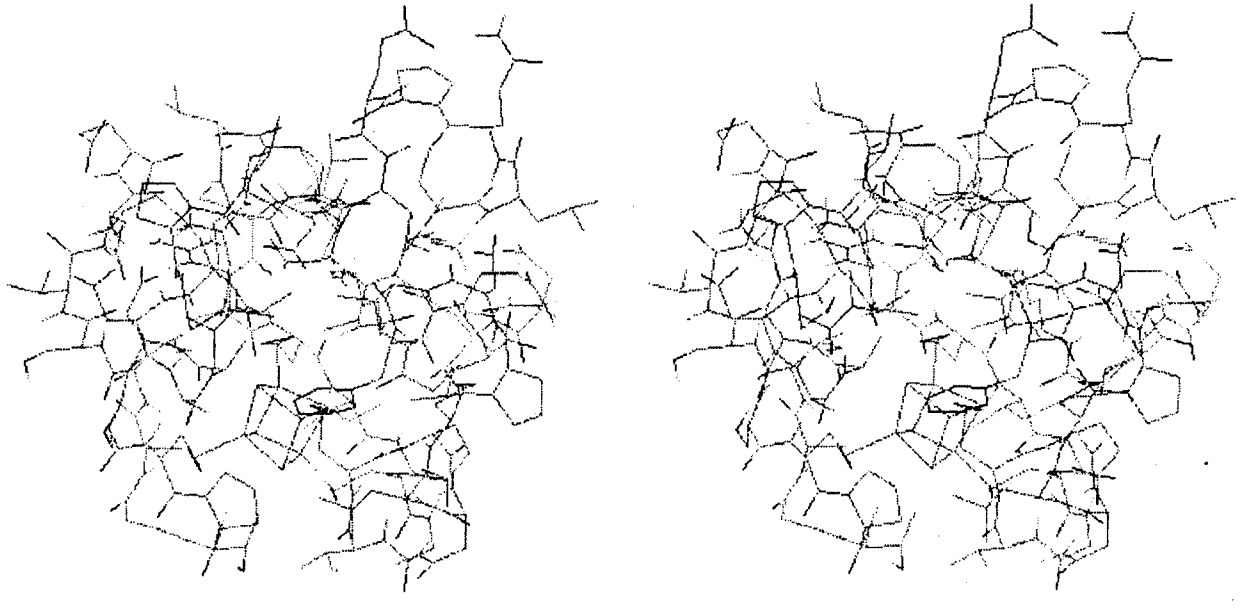

Fig. 2. Structure of the {*Thermotoga maritima*} Fd protein.

residues) and Fd of *Thermotoga maritima* (60 residues), nearly spherical proteins approximately 25 Å. (Figs. 1 and 2, respectively). In both proteins, the prosthetic groups are attached to the protein matrix by four covalent bonds between the iron atoms and the S_γ atoms of cysteine residues. The three-dimensional structures of the proteins have been published [7, 9].

Numerical modeling of protein dynamics was performed using a standard molecular dynamics technique, namely, the PUMA program developed in the Institute of Mathematical Problems in Biology [15, 16]. The protein model taken for numerical experiments, along with heavy atoms, contained. The starting coordinates of the heavy atoms were borrowed from the Brookhaven biomacromolecular structure database [17], and used to compute the coordinates of the hydrogens capable of H-bonding. The other hydrogen atoms were not considered explicitly, but their

existence was indirectly accounted for in the parameters of atom–atom interactions. The potential energy $U(r)$ determining the force field has components corresponding to deformations of valence bonds and angles, torsion angles, van der Waals and Coulomb noncovalent interactions, and H-bonding. The force interaction parameters and partial charges on the polypeptide chain atoms were taken from the set provided by Weiner *et al.* in 1984 [18]. Electrostatic interactions as well as van der Waals interactions were considered only for pairs of atoms spaced by no more than 10.5 Å. Interaction with the solvent was taken into account effectively, via collisions with virtual particles of the medium (collision thermostat).

The interaction parameters for the iron–sul-fur cluster atoms not available to date were chosen on the basis of quantum-chemical calculations [19, 20]. Partial charges on the Fe_4S_8 cluster atoms (adding the four sulfur atoms of cysteine residues) for the $2e^-$–$3e^-$ transition were derived from X-alpha computations for model compounds containing like clusters [19]. Partial charges on the cluster atoms for the $1e^-$–$2e^-$ transition were determined by adding to the atomic charges in state $2e^-$ the corresponding difference analogous to that for the $2e^-$–$3e^-$ transition. It has been demonstrated [21, 22] that some variations in charges within the framework of a given redox state are admissible inasmuch as they do not appreciably alter the spatial structure of the molecule. In state $3e^-$ (reduced ferredoxin) the overall charge of Fe_4S_8 and protein is $3e$, in state $2e^-$ (oxidized Fd or reduced HiPIP) it is $2e$, in state $1e^-$ (oxidized HiPIP) it is $1e$. Table 1 gives the partial charges on the iron–sulfur cluster atoms in different redox states. All three states were assumed to have the same equilibrium geometry and force field parameters of the clusters. In the unstressed state, the cluster was supposed to have T_d symmetry. The equilibrium values of the molecular parameters used in calculations are listed in Table 2. The force constants for all valence bonds and valence angles were obtained by quantum-chemical calculations for $Fe_4S_4(S_\gamma)_4H_4$ using the CNDO-S^2 method [23].

Table 1. Partial charges (e) on $Fe_4S_4(S_\gamma)_4$ atoms

Atom	$3e^-$	$2e^-$	$1e^-$
Fe	–0.101	–0.05	–0.001
S	–0.375	–0.275	–0.175
S_γ	–0.274	–0.175	–0.074

Table 2. Valent interaction parameters for the $Fe_4S_4(S_\gamma)_4$ complex

Bond or angle	Length (Å) or angle (deg)	Force constant, kcal mol^{-1} $Å^{-2}$, kcal mol^{-1} rad^{-2}
Fe – S	2.288	143
Fe – S_γ	2.25	201
Fe – S – Fe	73.74	32.5
S – Fe – S	104.12	25.4
S_γ – Fe – S	114.37	25.4
Fe – S_γ – C2	103.0	50

The X-ray structure of HiPIP [9] and the NMR-deduced structure of Fd [7] were taken as the initial ones. The initial rates were set at random in accordance with the Maxwell distribution. Preliminarily, the protein structures had been relaxed at constant temperature $T_0 = 300$ K; the duration of this relaxation stage was 100 to 300 ps. Then several MD paths of 100 ps at 300 K were obtained for every redox state possible for the given protein. The integration step was 0.3 fs, the mean frequency of collisions with the particles of the medium per atom was set at 5 ps^{-1}, and the characteristic time of interaction with the thermostat was set at $\tau_T = 1$ ps. The energy was averaged for the paths thus obtained, disregarding "too cold" or "too hot" paths.

RESULTS AND DISCUSSION

As follows from the data listed in Table 3, reduction of HiPIP leads to a 2.7 kcal/mol increase in the energy of repulsion within the iron–sulfur cluster proper; the energy of cluster interaction with the protein matrix also rises by 23.4 kcal/mol. Thus, a sum increment in energy of about 26 kcal/mol can be attributed to the increased negative charge on the cluster upon its reduction.

On the other hand, spectral measurements conducted with HiPIP in different redox states quite some time ago [24] have demonstrated that reduced HiPIP is oxidized in the light and reduced again upon cessation of illumination, i.e., the conversion from the oxidized to the reduced form takes place spontaneously, which is due to a negative change in free energy, $\Delta G = G_{red} - G_{ox}$.

Table 3. Different contributions to free energy for three redox states of HiPIP and the corresponding changes (kcal/mol)

Contribution	Oxidized state ($-1e^-$)	Reduced state ($-2e^-$)	Change $\Delta G = G_{red} - G_{ox}$	Superreduced state ($-3e$)	Change $\Delta G = G_{supr} - G_{red}$
Protein–cluster	110.29	73.32	–36.97	98.71	25.4
Protein–cluster	38.63	62.01	23.38	71.61	9.60
Cluster–cluster	5.50	8.24	2.74	12.18	3.94
Potential energy	154.42	143.56	–10.86	182.50	38.94
Kinetic energy	527.88	528.01	0.13	527.55	–0.46
Total energy	682.30	671.57	–10.73	710.05	38.48

Were the ΔG value determined only by the changes in the energy of interaction between the cluster and the protein globule and the own energy of the cluster, the overall change in free energy would have been positive (see above). However, the results given in Table 3 show that the change in the potential energy of the protein matrix itself is quite substantial and in this particular case more than compensates for the gain in the energy of Coulomb repulsion between the protein and cluster atoms and within the cluster. The potential energy of the protein matrix in the reduced form is almost 40 kcal/mol lower than that in the oxidized form. Hence the average energy of the system declines by about 11 kcal/mol.

It should be noted that for the *Rh. tenuis* HiPIP the redox potential or the mean standard transition potential $E_0 \approx +330$ mV corresponds to a $\Delta G \approx$ $\approx -23.06 \times 0.33 \approx -7.6$ kcal/mol. Our calculation gives a value some 30% higher, $\Delta G = -10.73$ kcal/mol. This discrepancy appears to be due to the interaction of the protein matrix and the cluster with the water surroundings, whereas the model considered at this stage does not take into account the influence of solvent. That is, the presence of water shifts the potential by some 4 kcal/mol toward the oxidized form. It is known that in HiPIP the iron–sulfur cluster is buried practically in the middle so as to be inaccessible for water molecules (there are data that no deuterium exchange takes place even after three-day exposure [25]). Therefore, the influence of solvent in this case should not be as pronounced as, for instance, in the other iron–sulfur protein tested, ferredoxin (see below).

We also calculated the average total energy for "superreduced" HiPIP in which there are three electrons on the iron–sulfure cluster. Such a state does not occur metabolically, and is experimentally attained in the presence of dimethyl sulfoxide [27]. The superreduced state for HiPIP has been thought to be unstable [25].

As evident from Table 3, the mean energy in the superreduced state is higher than that in the two-electron state, and the mean standard transition potential in this case is –1670 mV. This is in fair agreement with the values for synthetic $Fe_4S_4(SR)_4$ clusters in various solvents [27] and with the theoretical prediction of anomalously negative redox potentials for different HiPIPs in superreduced state [22]. According to these data, in nonpolar solvents $E_0 \approx -1200$ mV, and it changes to some –700 mV in water-containing solvents.

With ferredoxin, the situation proves to be different. The calculations give a higher average energy for the oxidized two-electron state of Fd versus the reduced state with three electron on the iron–sulfur cluster. However, spontaneous oxidation has been

Table 4. Different contributions to free energy for two redox states of Fd and the corresponding changes (kcal/mol)

Contribution	Oxidized state ($-2e^-$)	Reduced state ($-3e^-$)	Change $\Delta G = G_{red} - G_{ox}$
Protein–cluster	–37.08	– 54.10	– 17.02
Protein–cluster	81.04	90.42	9.38
Cluster–cluster	7.89	12.94	5.05
Potential energy	51.84	49.25	–2.59
Kinetic energy	501.39	501.44	0.05
Total energy	553.23	550.69	– 2.54

observed experimentally for this protein [26], which means that the free energy in the oxidized state of Fd must be lower than the free energy in the reduced state. As shown in Table 4, in the reduced state the potential energy of the protein matrix harboring the iron–sulfur cluster is lower than the potential energy of the protein matrix in the oxidized Fd by 17 kcal/mol. On the other hand, reduction is attended with a rise in the energy of repulsion of the cluster atoms both between themselves (by about 5 kcal/mol) and from the atoms of the protein chain (by 9.4 kcal/mol).As in the case of HiPIP, the protein matrix tends to counterbalance the positive contribution to the potential energy of Coulomb repulsion associated with the increase in the sum charge of the same sign on the cluster. If we disregard the influence of surrounding water, such compensation is overwhelming so that the average energy in the reduced state appears to be 2.5 kcal/mol lower than that in the oxidized state, which is inconsistent with the experimental data. It should be borne in mind that in the ferredoxin molecule the iron–sulfur cluster is close to the surface, which is corroborated by the data on the deuterium exchange rate [25]. The solvent effect on the cluster in this protein is probably substantial and promotes a shift of the equilibrium toward the oxidized form of Fd. As noted above, in the HiPIP case this effect proves to be insufficient because of isolation of the iron–sulfur cluster from solvent as well as because of the much greater contribution of the protein matrix into the energy favoring the reduced form.

CONCLUSIONS

The results of molecular dynamics calculations show that for the HiPIP molecule the value of redox potential (or ΔG) is largely determined by intraprotein interactions. The contribution of the peptide matrix to the heat effect of the reaction is definitive, shifting the equilibrium toward the reduced form. The HiPIP molecule can also exist in superreduced state with a markedly negative standard transition potential.

In the case of ferredoxin, the proximity of the iron–sulfur cluster to the protein surface alters essentially the energy aspects of the redox reaction. The contribution of the protein matrix is also appreciable, though it is 2–2.5 times less that for HiPIP where the cluster is buried in the middle of the globule. In the absence of water, the equilibrium for Fd should be slightly biased toward the reduced form. However, the presence of solvent molecules radically affects the sign of the reaction heat, and in reality the equilibrium proves to be shifted toward the oxidized form of ferredoxin.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research (projects nos. 98-04-48479 and 00-04-49302), INTAS (990281), and the Ministry of Education (220-7).

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