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CONTRIBUTION OF THE EXCHANGE INTERACTIONS TO THE REDOX PROPERTIES OF THE [2Fe-2S] FERREDOXINS

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It is shown that in the [2Fe-2S] ferredoxins, the exchange interactions between the two iron atoms of the redox cluster provide a relative stabilization of the oxidized state. Compared to the uncoupled situation, this leads to a significant lowering of the redox potential which can be larger than 100 mV. This effect could be one of the main origins of the low potential of these ferredoxins, compared to the potential of rubredoxins.

Introduction

The iron-sulfur proteins are involved as electron carriers in numerous biological processes. They have been thoroughly characterized by a full body of physicochemical investigations [1]. The redox potential of those proteins belonging to the [2Fe-2S] group is usually more negative than the potential of the rubredoxins, whose active site is composed of only one iron center (Table I). A similar trend holds for the synthetic analogues of these two types of redox clusters: in a dimethylformamide solution, $Fe(S_2\text{-}o\text{-}xyl)_2^-$ is reduced at -1.02 V compared to the standard calomel electrode [18], whereas under the same conditions $Fe_2S_2(S_2\text{-}o\text{-}xyl)_2^{2-}$ is reduced at -1.49 V [19]. The generality of this negative shift suggests that it could mainly originate from the peculiar structure of the binuclear cluster, which is represented in Fig. 1. This is supported by two experimental facts: (i) the existence in desulforedoxin of a new redox cluster, where only one iron atom is coordinated to four cysteine groups. The spectroscopic properties suggest that the geometry of this cluster is significantly different from that of the rubredoxins; nevertheless, their redox potential $E^0 \approx -35$ mV falls in the same range [20]; (ii) the effect of the substitution of selenium for labile sulfur in the [2Fe-2S] ferredoxins: although this substitution induces important spectral shifts (see Refs. 4–11 cited in Ref. 21), the redox potential is weakly affected (Table I).

In the electrostatic model proposed by Kassner and Yang [22,23] to explain the differences between the redox potentials of the rubredoxins and the [2Fe-2S] ferredoxins, the internal energy of the redox clusters is calculated by a summation over

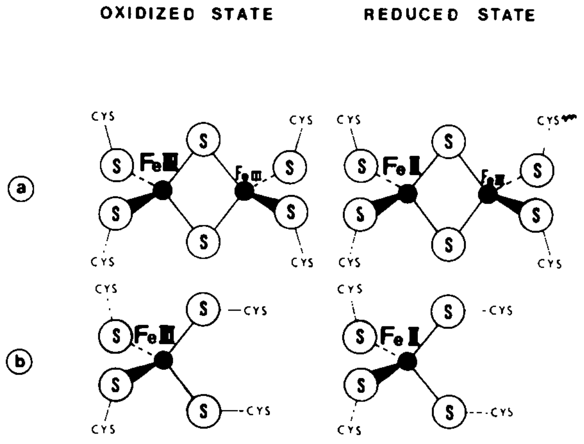

Fig. 1. Redox clusters of (a) the [2Fe-2S] ferredoxins and (b) the rubredoxins, in the oxidized and the reduced states.

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TABLE I

MIDPOINT REDOX POTENTIALS OF THE RUBREDOXINS AND THE [2Fe-2S] FERREDOXINS COMPARED TO A STANDARD HYDROGEN ELECTRODE, pH≈7, 25°C

Protein	E^0 (mV)	Ref.
Rubredoxins		
Clostridium pasteurianum	−57	2
Pseudomonas oleovorans	−37	3
Desulfovibrio gigas	+6	4
Desulfovibrio salexigens	−31	4
Desulforomonas acetoxidans	−46	4
Clostridium thermoaceticum 1	−27	5
Clostridium thermoaceticum 2	+20	5
[2Fe-2S] ferredoxins		
Plants and algae	−310 to −455	6
Parsley	−416	7
Adrenal glands	−270	8
Pseudomonas putida	−235	9
Azotobacter vinelandii II	−225	10
Clostridium pasteurianum	−300	11
Agrobacterium tumefaciens	−220	12
Escherichia coli	−380	13
Halobacterium halobium	−345	14
Halobacterium from the Dead Sea	−345	15
Selenium-substituted [2Fe-2S] ferredoxins		
Parsley	−365	7
Adrenal glands	−288	16
Pseudomonas putida	−245	17

all the coulombic interactions between the iron and sulfur ions [22,23].

In the [2Fe-2S] ferredoxins, one has also to consider the large antiferromagnetic exchange component induced by the electrostatic interactions between the iron centers. It is shown in the present paper that this exchange component provides a relative stabilization of the oxidized state, and thus a lowering of the redox potential whose magnitude can be estimated to be about 100 mV.

Calculation of the stabilization provided by the exchange interactions in a binuclear cluster

In the [2Fe-2S] ferredoxins, the main energetic variations that occur upon reduction are located at the reducible center, where the iron atom undergoes a valence state change Fe(III) → Fe(II), as in rubredoxins (Fig. 1). However, in the binuclear cluster, the strong antiferromagnetic exchange interactions which are allowed by the vicinity of the nonreducible Fe(III) center provide an extra stabilization. This stabilization cannot be directly deduced from the Heisenberg hamiltonian $-2J\vec{S}_1 \cdot \vec{S}_2$, which was used to measure the exchange interactions in the [2Fe-2S] ferredoxins. This hamiltonian only gives the energy differences between the spin multiplets. However, it is possible from an analysis of the mechanism of the exchange interactions to express the stabilization as a function of the parameter J.

The J value actually includes two different antiferromagnetic contributions:

$$J = J_1 + J_2 \qquad J_1 < 0, J_2 < 0$$

The contribution J_1 results from the effect of the interaction hamiltonian within the ground configuration of the binuclear cluster, whereas J_2 results from the interaction between the ground configuration and the charge-transfer excited configuration [24].

In the configuration interaction mechanism, the energy of the highest multiplicity state characterized by:

$$S_{\max} = S_1 + S_2$$

is identical with the energy in the absence of interaction [25]. The stabilization of the ground level characterized by:

$$S_{\min} = S_1 - S_2 > 0$$

can then be calculated from the Heisenberg hamiltonian, and is equal to $4|J_2|S_1S_2 + 2|J_2|S_2$

In the mechanism which leads to J_1, the state characterized by $S_{\max}$ is destabilized by an amount $4|J_1|S_1S_2$ (Appendix). The stabilization of the ground level is then equal to $2|J_1|S_2$.

Considering both effects, the net stabilization E of the ground level can be written:

$$E = 2[|J_1| + |J_2|]S_2 + 4|J_2|S_1S_2 \tag{1}$$

The stabilization E can be estimated in the oxidized and in the reduced states from the values $J(\text{ox}) \approx -180\ \text{cm}^{-1}$ and $J(\text{red}) \approx -80\ \text{cm}^{-1}$ [26–31] which were measured for different [2Fe-2S] plant ferredoxins. In fact, its effective value depends in each case upon the relative importance of J_1 and

TABLE II

CONTRIBUTION OF THE EXCHANGE INTERACTIONS TO THE RELATIVE STABILIZATION $\Delta E = E(\text{ox}) - E(\text{red})$ OF THE OXIDIZED FORM OF THE [2Fe-2S] CLUSTERS, FROM EQN. 1 IN THE TEXT

The values of E and ΔE were calculated in the two extreme cases $J = J_2$ ($J_1 = 0$) and $J = J_1$ ($J_2 = 0$) by using the exchange values measured for the plant ferredoxins: $|J(\text{ox})| \approx 180\ \text{cm}^{-1}$ and $|J(\text{red})| \approx 80\ \text{cm}^{-1}$.

	E(ox)	E(red)	$\Delta E = E(\text{ox}) - E(\text{red})$
$J_1 = 0$	5400 cm^{-1}	1920 cm^{-1}	3480 cm^{-1} = 0.430 eV
$J_2 = 0$	900 cm^{-1}	320 cm^{-1}	580 cm^{-1} = 0.072 eV

J_2. The ratio J_1/J_2 is not known but, by setting alternatively $J_1 = 0$ and $J_2 = 0$, we obtain, respectively, an upper and a lower limit for the energy difference $\Delta E = E(\text{ox}) - E(\text{red})$ which represents the relative stabilization of the oxidized form of the binuclear cluster provided by the exchange interaction.

It is clear from the results given in Table II that even for a weak contribution of the J_2 mechanism, the strong antiferromagnetic exchange interactions lead to a significant lowering of the redox potential.

Discussion

In the [2Fe-2S] ferredoxins, one half of the redox cluster is constituted in both oxidation states by an Fe(III) center surrounded by a distorted tetrahedron of sulfur ligands (Fig. 1). The electronic states of this center appear almost insensitive to the oxidation level of the protein, as observed through its Mössbauer parameters [27,32,33], ultraviolet-visible [34] and near-infrared [35] absorption spectra. It is therefore expected that the redox energy contribution of this nonreducible center is weak. The free energy variations that occur upon reduction are mainly localized at the reducible center, where an Fe(III) → Fe(II) valence change takes place.

On the other hand, from comparison of the Mössbauer [20,27,32,33,36], ultraviolet-visible [34], MCD [37,38] and infrared [35,39] spectra, the reducible center of the [2Fe-2S] ferredoxins and the redox cluster of the rubredoxins appear to be very similar in both oxidation states. This suggests that the two redox changes represented in Fig. 1 are closely related. Accordingly, the exchange interactions which lead to a lowering of the redox potential of the [2Fe-2S] ferredoxins of about 100 mV would contribute significantly to the difference between the redox potentials of these ferredoxins and the rubredoxins.

The aim of this work was to put in evidence one particular component of the free energy redox variation in the [2Fe-2S] ferredoxins, namely the contribution due to the exchange interactions. The overall value of the redox potential is determined by many other factors, such as the specific nature of the protein medium and its solvent. For example, it is noteworthy that the redox potential of some proteins belonging to the mitochondrial electron-transfer system, and considered as [2Fe-2S] ferredoxins is more positive than those of rubredoxins, contrary to the soluble ferredoxins listed in Table I [42].

The great similarity of the structural and spectroscopic properties of rubredoxins and [2Fe-2S] ferredoxins provides a basis for the comparison of their redox properties. At the present time, such a similarity has not been established between rubredoxins and [3Fe-3S] or [4Fe-4S] ferredoxins, and it is not possible to discuss the thermodynamic importance of the exchange interactions for these proteins.

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Appendix

Destablization energy of the highest multiplicity state for a cluster of two paramagnetic centers coupled by antiferromagnetic exchange interactions

Consider two paramagnetic centers A and B characterized by their spin S_A and S_B. On each center, the unpaired electrons are localized in magnetic orbitals noted:

$$\{\Phi_{i,A}\}\ i=1,\ldots n_A \quad n_A=2S_A$$

$$\{\Phi_{j,B}\}\ j=1,\ldots n_B \quad n_B=2S_B \quad n_A \leqslant n_B$$

These two bases are chosen to be orthonormalized:

$$\langle \Phi_{i,A}|\Phi_{j,A}\rangle = \langle \Phi_{i,B}|\Phi_{j,B}\rangle = \delta_{ij}$$

We suppose that each orbital of one center can overlap with only one orbital of the other center, and we note

$$S_i = \langle \Phi_{i,A}|\Phi_{i,B}\rangle$$

The antiferromagnetic exchange interaction between A and B results from the sum of monoelectronic hamiltonians [40]

$$H = \sum_{i=1}^{n_A+n_B} h(i)$$

Within the ground configuration (A, B), H leads to an energy level scheme which can be described by the Heisenberg hamiltonian $-2J_1\vec{S}_A \cdot \vec{S}_B$ with [40]:

$$J_1 = \frac{2}{n_A n_B} \sum_{i=1}^{n_A} \frac{S_i(\beta_i - \alpha_i S_i)}{1 - S_i^4} \qquad \text{(A1)}$$

where

$$\alpha_i = \tfrac{1}{2}(\alpha_{iA} + \alpha_{iB})$$

$$\alpha_{iA} = \langle \Phi_{i,A}|h|\Phi_{i,A}\rangle$$

$$\alpha_{iB} = \langle \Phi_{i,B}|h|\Phi_{i,B}\rangle$$

$$\beta_i = \langle \Phi_{i,A}|h|\Phi_{i,B}\rangle$$

The state $|\Psi>$ of maximum spin $S = S_A + S_B$ is represented by the single Slater determinant:

$$|\Psi> = \frac{1}{\sqrt{(n_A+n_B)!}} |\Phi_{1,A}\Phi_{1,B}\cdots\Phi_{n_A,A}\Phi_{n_A,B}\cdots\Phi_{n_B,B}|$$

The energy of this state in the presence of H is calculated by using the method given by Slater [41]:

$$E(\Psi) = \frac{\langle\Psi|H|\Psi\rangle}{\langle\Psi|\Psi\rangle} = 2\sum_{i=1}^{n_A} \frac{\alpha_i - \beta_i S_i}{1 - S_i^2} + \sum_{i=n_A+1}^{n_B} \alpha_{iB}$$

This expression can be written

$$E(\Psi) = E_c + E_e$$

with

$$E_c = 2\sum_{i=1}^{n_A} \alpha_i + \sum_{i=n_A+1}^{n_B} \alpha_{iB}$$

$$E_e = -2\sum_{i=1}^{n_A} \frac{S_i(\beta_i - \alpha_i S_i)}{1 - S_i^2}$$

E_c and E_e are, respectively, the coulombic and exchange contributions of the hamiltonian H. Making the usual assumption $S_i^2 \ll 1$, we can express the exchange part E_e in terms of J_1 (Eqn. A1):

$$E_e = -n_A n_B J_1 = 4\, S_A S_B |J_1|$$

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