ACCOUNTS
of chemical research

Unveiling the Details of Electron Transfer in Multicenter Redox Proteins

CATARINA M. PAQUETE AND RICARDO O. LOURO*
Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República (EAN), 2780-157 Oeiras, Portugal

RECEIVED ON MARCH 7, 2013

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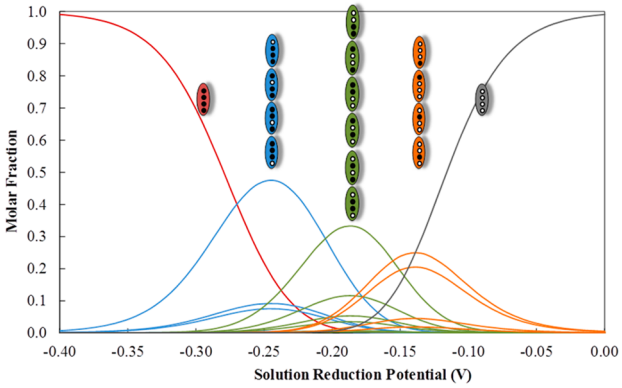

Metalloproteins modulate the intrinsic properties of transition metals to achieve controlled catalysis, electron transfer, or structural stabilization. Those performing electron transport, redox proteins, are a diverse class of proteins with central roles in numerous metabolic and signaling pathways, including respiration and photosynthesis. Many redox proteins have applications in industry, especially biotechnology, making them the focus of intense research. Redox proteins may contain one or multiple redox centers of the same or a different type. The complexity of proteins with multiple redox centers makes it difficult to establish a detailed molecular mechanism for their activity. Thermodynamic and kinetic information can be interpreted using the molecular structure to elucidate the protein's functional mechanism.

This Account reviews experimental strategies developed in recent years to determine the detailed thermodynamic properties of multicenter redox proteins and their kinetic properties during interactions with redox partners. These strategies allow the discrimination of thermodynamic and kinetic properties of each individual redox center. The thermodynamic characterization of the redox transitions results from the combined analysis of data from NMR and UV-visible spectroscopy. Meanwhile, the kinetic characterization of intermolecular electron transfer comes from stopped-flow spectrophotometry. We illustrate an application of these strategies to a particular redox protein, the small tetraheme cytochrome from the periplasmic space of *Shewanella oneidensis* MR-1. This protein is a convenient prototype for developing methods for the detailed analysis of multicenter electron transfer proteins because hemes have strong UV-visible absorption bands and because heme resonances have exquisite discrimination in NMR spectra. Nonetheless, the methods are fully generalizable.

Ultimately, this Account highlights the relevance of detailed characterization of the thermodynamic and kinetic properties of redox proteins. These properties are responsible for the directionality and specificity of the electron transfer process in bioenergetic pathways; a more thorough characterization of these properties should allow better-designed proteins for industrial applications.

Electron transfer in redox proteins

Long-range electron transfer events in biology occur through the outer-sphere mechanism where the inner coordination shells of both the oxidant and the reductant remain intact in the transition state and the electron is forced to move from one redox center to the other. This phenomenon can be described in the frame of Marcus' theory of electron transfer.[1] This theory can be formulated in simple terms using experimentally accessible parameters relevant for biological electron transfer:[2]

$$\log_{10} k_{et} = 13 - 0.6(R - 3.6) - 3.1(\Delta G + \lambda^2)/\lambda \quad (1)$$

Here R is the electron transfer distance, ΔG is the driving force, and λ is the reorganization energy. Equation 1

Published on the Web 08/28/2013 www.pubs.acs.org/accounts
10.1021/ar4000696

SCHEME 1

$$\underbrace{A^{ox} + D^{red} \overset{K_1}{\rightleftharpoons} \left[A^{ox} - D^{red}\right] \overset{K_x}{\longrightarrow} \left[A^{ox} - D^{red}\right]^*}_{\text{Encounter complex}} \overset{k_{et}}{\longrightarrow} \underbrace{\left[A^{red} - D^{ox}\right] \overset{K_2}{\rightleftharpoons} A^{red} + D^{ox}}_{\text{Departure complex}}$$

shows that for distances that range between 4 and 14 Å, the reactions proceed within time scales that are typically faster than millisecond, which are adequate to support the physiological catalytic rates of enzymatic reactions.[2] Fast electron transfer over larger distances is supported by arrangements of the redox centers in ways that split the distance between the electron donor and the final electron acceptor into a series of short steps.[3]

Electron transfer between proteins that are not permanently bound depends on approach by diffusion, followed by random collisions. Electrostatic forces contribute significantly to the initial phase of protein−protein complex formation.[4] After the actual electron transfer event, the dissociation of the protein−protein complex finalizes the electron transfer reaction (Scheme 1).

Here, D is the electron donor and A the electron acceptor, K_1 is the binding constant of the protein−protein complex, K_2 is the dissociation constant for the products of electron transfer, and k_{ET} is the electron transfer rate constant. In some cases, a non-electron-transfer step (K_x), such as protein rearrangements or conformational changes within the complex, may be required to optimize or activate the system for electron transfer.[5]

In biological systems, fast turnover is necessary to minimize constraints to the electron transfer process. In conditions of fast turnover, only a small fraction of the collisions will bring the redox proteins into the proper distance and orientation that are conductive to electron transfer.[4,6] When multicenter redox proteins interact and several of the centers have substantial surface exposure, multiple options exist for which centers actually donate the electrons and which actually receive them. Identification of the centers that contribute to the intermolecular electron transfer and the fate of each electron transferred within the acceptor requires knowledge of the detailed thermodynamic and kinetic properties of the various redox centers in each partner.

Thermodynamic Properties of Multicenter Redox Proteins

In proteins with only one redox center, its reduction potential can be obtained directly by the application of the Nernst equation, where n is the number of electrons involved in the reaction:

$$E = E^\circ + \frac{RT}{nF} \ln \frac{[ox]}{[red]} \quad (2)$$

In multicenter redox proteins the information obtained is conditioned by the capacity of the experimental technique to discriminate the individual centers. When the multiple centers have identical experimental signal, the sum of x Nernst equations, with x being the number of redox centers, provides one reduction potential for each center.[7] This is the simplest analysis that can be performed on such data that does not resort to structural information but has the implicit assumption that the multiple centers do not interact with each other. For this *noninteraction model* to be valid, the redox centers must be placed far away from each other within the low dielectric protein environment. The distance beyond which electrostatic interactions typically become smaller than 5 mV is estimated to be approximately 17 Å (metal-to-metal),[8] which is larger than what is observed between neighboring centers in the vast majority of multicenter redox proteins.

An alternative analysis involves the definition of sequential midpoint reduction potentials for each electron that can be exchanged with the protein.[9,10] This *macroscopic model* defines a reduction potential for each electron, with no implicit assumptions about the structure of the redox protein. However, these reduction potentials cannot be formally assigned to specific redox centers in the structure and for that reason are called macroscopic. The *macroscopic* and the *noninteracting* models define the same number of parameters from the experimental data, but the physical meaning of these parameters is different and therefore their values cannot be compared.

If a protein with multiple redox centers displays distinguishable experimental signatures that allow their titrations to be monitored individually, a more detailed analysis can be performed. This *microscopic model* defines the reduction potential of each center as well as the interactions between nearby redox centers. Such interactions modify the shape or position of the titration curve or both for each redox center. For interacting centers with similar reduction potentials the slope of the titration curve is different from that of a Nernst curve. Flatter slopes indicate repulsive interactions (negative

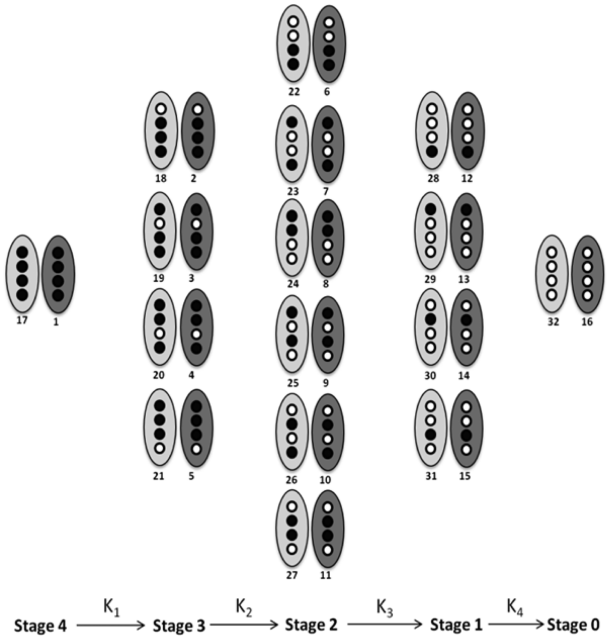

FIGURE 1. Schematic representation of the microscopic states for a multicenter redox protein with four redox centers and one acid–base center. Light- and dark-gray ovals represent deprotonated and protonated microstates, respectively. Black and white dots represent reduced and oxidized redox centers, respectively. Redox stages grouping states with the same number of oxidized redox centers are arranged in columns. States are arbitrarily numbered from 1 to 32.The five sequential stages of oxidation linked by four macroscopic redox transitions are indicated in the bottom, showing the direction of oxidation.

cooperativies), and steeper slopes indicate attractive interactions (positive cooperativities). The observed difference provides a measure of the magnitude of the interactions.[11] For two interacting centers with very different reduction potentials, the titration of one center is effectively finished before the other begins. In these conditions, the titration curves have a Nernst shape but the position is shifted by the magnitude of the interaction. Hence, determination of the value of the interaction requires mutations that affect the potentials without disturbing the structure.[12] Modifying the chemical conditions such as the pH at which the measurements are performed, enables the parsing of homotropic and heterotropic cooperativities. A phenomenon of great biological consequence that can also be analyzed this way is the heterotropic thermodynamic interaction between electrons and protons, known as the redox-Bohr effect.[13,14]

For example, for a protein with four redox centers and one acid–base center, five stages of reduction can be defined. These are numbered 0 to 4 according to the number of oxidized hemes in the microstates included in each stage. Each redox stage includes the protonated and deprotonated microstates of the acid–base center (Figure 1). This description is general and

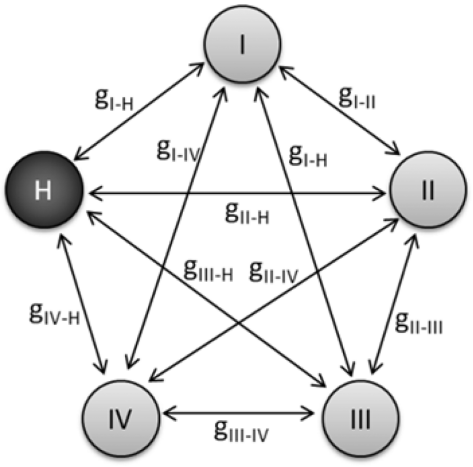

FIGURE 2. Schematic representation of the interaction network present in a multicenter redox protein with four redox centers (I–IV) and one acid–base center (H). The interacting energies between the various centers are represented as g_i and g_{iH}, where i is the redox center.

can be scaled to any number of redox and acid–base centers necessary to describe the protein under study.

For any experimental condition of pH and reduction potential, the population of these 32 microstates can be defined by 15 thermodynamic parameters (Figure 2): four reduction potentials, one pK_a for the acid–base center, six homotropic interactions between pairs of redox centers, and four interactions between the redox centers and the acid–base center.[15]

Analyzing such a system using a set of interrelated Nernst equations becomes extremely cumbersome from the mathematical point of view, in particular for the cases that display heterotropic interactions. An approach that considers the free energy differences between states provides a more streamlined mathematical formulation.[15]

Using Figure 1 as example and considering the reduced and protonated state of the protein as reference ($G_1 = 0$), the free energy of every state relative to the reference state can be determined. For a redox state that has only one center oxidized, this is given by

$$G_x = g_i + FE \tag{3}$$

where x is the arbitrary numbering of the state, i is the redox center, g_i is the energy of oxidation of center i, and E is the redox potential of the solution. When various redox centers are oxidized, the interaction energy between them has to be considered, and therefore the energy of the final state relative to the reference is given by

$$G_x = \sum g_i + \sum I_{ij} + nFE \tag{4}$$

with $\sum g_i$ and $\sum I_{ij}$ being the sum of oxidation energies of the various centers and the sum of the interaction energy between each pair of redox centers, respectively, and n being number of centers oxidized. If the acid−base center becomes deprotonated and one of the redox centers is oxidized then the relative free energy is given by

$$G_x = g_i + g_{\mathrm{H}} + I_{i\mathrm{H}} + FE + 2.3\frac{RT}{F}\,\mathrm{pH} \tag{5}$$

The resulting values can be related with the reduction potential for any transition between states using the thermodynamic relationship

$$\Delta G = -nF\Delta E \tag{6}$$

Equations 3−5 define energies for all states relative to the reference state. This information can then be used to determine their populations relative to the population of the reference state using the Boltzmann equation:

$$P_x = \exp\left(-\frac{F}{RT}G_x\right) \tag{7}$$

This analysis can be scaled to describe any number of centers as necessary for the protein being studied, including more than one acid−base center, if required by the experimental data.[16] For n centers, the number of parameters that must be defined by the experimental data are given by $n + n(n-1)/2$, when pairwise interactions are considered.[11] This is achieved computationally by using algorithms of nonlinear fitting of the model to all experimental data. Once an acceptable minimum has been found, the diagonal elements of the covariance matrix are checked to ensure that the calculated parameters are well-defined by the experimental data. However, the thermodynamic properties only provide information on the range of states accessible to the proteins under study. To know which states and transitions truly play a functional role during oxidation or reduction by redox partners, kinetic information is required at the same level of detail.

Kinetics of Intermolecular Electron Transfer in Multicenter Proteins

In biological systems, interprotein redox reactions typically have low driving forces and often occur in the millisecond time scale. When these reactions are not gated by other processes, a collisional description can be considered. This description assumes fast equilibrium for the encounter and departure complexes (see Scheme 1), making the electron transfer process the rate-determining step, in the absence of slower rearrangements within the protein complex.[17]

When one of the partners is a multicenter redox protein, a detailed characterization of the reduction or oxidation requires that an electron transfer process is considered for each possible transition between microstates of different redox stages (Figure 1). A description of the reduction or oxidation of a multicenter protein with four redox centers requires the definition of 64 electron transfer microscopic steps (all possible one-electron transitions between stages that involve microstates 1−16 or that involve microstates 17−32 in Figure 1). This will clearly overwhelm any conceivable experimental data set, and therefore a simplification is required. This can be achieved by establishing pseudo-first-order conditions using a large excess of the redox partner. In these conditions, 32 irreversible microscopic electron transfer steps between stages must be defined. These are still too many to be extracted directly from kinetic experiments, and therefore extra information must be obtained from elsewhere.

The short distance typically found between redox centers within a multicenter protein makes intramolecular equilibration of the electrons according to the reduction potentials of the centers faster than intermolecular electron transfer from the donor or to an acceptor. Therefore, equilibrium populations are established among the microscopic states within each stage between successive intermolecular electron transfer events. As a consequence, the description of the four-center protein collapses to a simple kinetic scheme of four consecutive steps of one-electron intermolecular transfer (Figure 1, bottom), with each step defined by a macroscopic rate constant. This reduces the number of parameters to a value that is equal to the number of redox centers and therefore can be experimentally extracted. However, these parameters are macroscopic and cannot be directly assigned to individual centers within the protein. To achieve this assignment, the value of the rate constant of each macroscopic step ($K_{\mathrm{step}(1-4)}$) is parsed into the contributions of all the microscopic rate constants (k_i^j; i, the center being reduced or oxidized, and j, the other centers already reduced or oxidized) of the transitions that participate in the step (Figure 3). Each contribution is weighed according to the fraction of the populations of the starting stage (χ_i^j), which are known from the detailed thermodynamic characterization:

$$K_{\mathrm{step}(1-4)} = \sum_i^n \chi_i^j k_i^j \tag{8}$$

Given that the change of the free energy for each transition is also known from the detailed thermodynamic characterization, the differences in the electron transfer rate for a

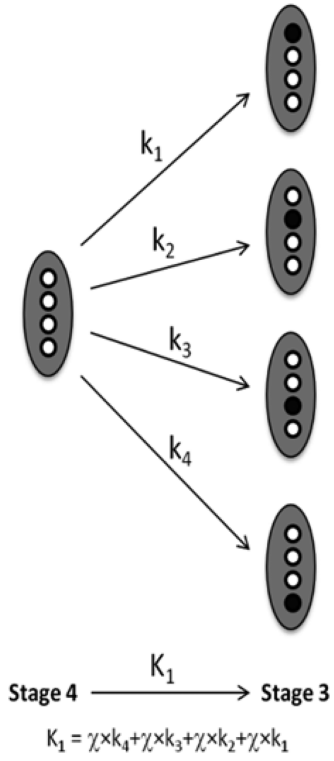

FIGURE 3. Parsing of the macroscopic rate constants, taking into account the fraction of the population of the starting stage and the microscopic rate constants that contribute to that particular step. The transition from stage 4 to stage 3 is illustrated, and for this reason no superscripts are indicated because there are no other centers already reduced.

specific center in different steps can be interpreted within the framework of Marcus' theory of electron transfer.[1] In these conditions, the microscopic rate constant for a particular reduction microstep of a multicenter protein can be parsed into a reference rate constant for each center (k_i^0) that accounts for the rate of the reaction at zero driving force and an exponential factor that accounts for the driving force associated with each microstep:[17]

$$k_i^j = k_i^0 \exp\left[\frac{e_i F}{2RT}\left(1 + \frac{e_D F}{\lambda} - \frac{e_i F}{2\lambda}\right)\right] \quad (9)$$

The value of the driving force depends solely on the thermodynamic parameters of the system given by e_i and on the reduction potential of the electron donor (e_D). A similar equation can be defined for the case of oxidation reactions, where e_A is the reduction potential of the electron acceptor:[18]

$$k_i^j = k_i^0 \exp\left[\frac{e_i F}{2RT}\left(-1 + \frac{e_A F}{\lambda} - \frac{e_i F}{2\lambda}\right)\right] \quad (10)$$

This analysis contains the implicit assumption that the environment around each redox center does not undergo significant modifications throughout the titration of the protein so that a single value of the reorganization energy is representative for all transitions. This assumption must be treated with care for those cases where redox proteins are known to display significant redox linked conformational changes.[17] This analysis also contains the implicit assumption that electrostatic interactions between the redox partners play a minor role in the rate of electron transfer.[19]

Application of this strategy has the merit of retaining a number of parameters to be defined from the kinetic data equal to the number of redox centers. Therefore, it can be applied even when the redox centers cannot be discriminated experimentally. Furthermore, the reference rate constants (k_i^0) are intrinsic to each center and depend only on structural factors. This information enables the definition of the role of each redox center in the overall process of intermolecular electron transfer.

A Case Study: Multiheme Proteins

Heme-containing proteins are among the most versatile of metalloproteins with respect to their physiological functions, having gained the designation of Swiss army-knife of iron.[20] The hemes in *c*-type cytochromes are covalently attached to the polypeptide through amino-acid sequences that are typically -CXXCH-. In cytochromes containing various hemes, they are numbered with roman numbers according to their order of attachment to the polypeptide chain. The combination of differences in the polypeptide chain, the number of bound hemes, and their axial coordination allow these proteins to exhibit an impressive range of biological functions.[21−23] These include electron transfer, ligand binding for transport, signal transduction, and enzyme activity regulation.[24]

Genomic surveys are revealing hundreds of genes encoding hypothetical *c*-type cytochromes, with several organisms containing more than 40.[25] In addition, the notion that multiheme *c*-type cytochromes are the class of proteins with the largest number of redox centers per polypeptide is becoming more firmly established.[24] Currently, the putative record holder is a gene product from the Gram positive bacterium *Thermincola potens* JR that displays 57 canonical heme-*c* binding motifs.[26] The structural characterization of this class of proteins lags far behind, and the most complex monomeric cytochrome characterized to date is the hexadecaheme cytochrome from *Desulfovibrio*.[27] The impressive diversity of physiological functions of cytochromes has been attributed to the thermodynamic and kinetic properties of their redox centers, which in turn are modulated by the

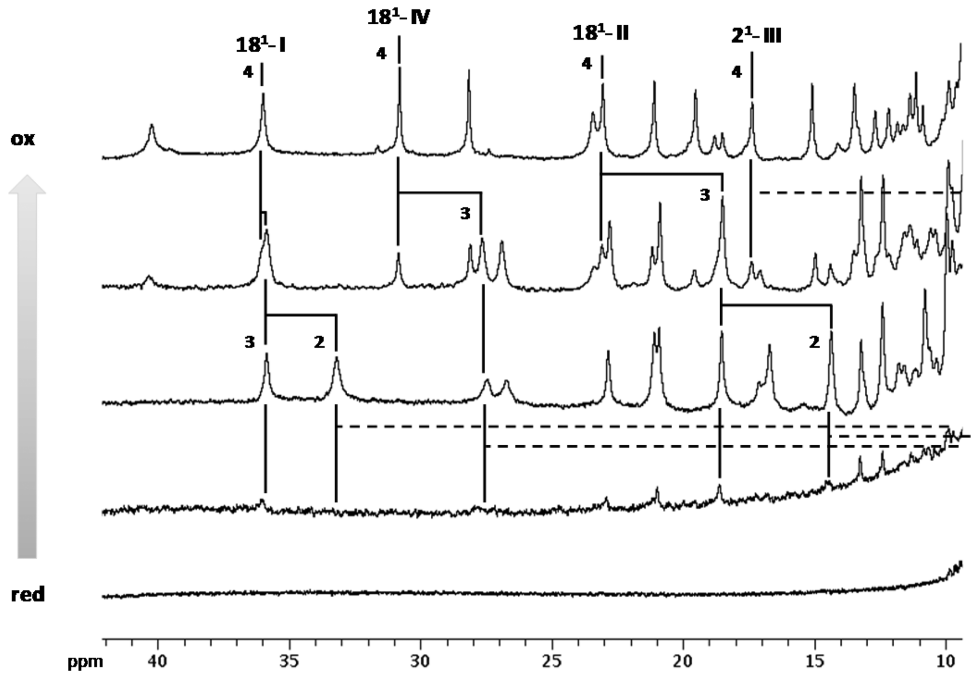

FIGURE 4. Low-field region of the 1D-NMR spectra of STC from *S. oneidensis* MR-1 in the reduced, partially reduced, and oxidized forms. The methyl groups are identified using the IUPAC-IUB nomenclature for hemes. The Roman numeral identifies the heme (hemes I–IV), and the number close to each line corresponds to the redox stage (numbered from 0 to 4 as in Figure 1). Lines connect signals belonging to the same methyl in different redox stages of the protein. Dashed lines indicate a connection to a position in the diamagnetic region of the spectrum.

environment provided by the protein. While thermodynamic knowledge is essential to identify the possible electron transfer pathways, it is the kinetic properties that define whether a particular rate is sufficiently fast for intermolecular electron transfer to occur in the lifetime of a transient protein–protein complex.

One of the most powerful techniques for studying multiheme cytochromes is Nuclear Magnetic Resonance Spectroscopy (NMR) which allows the spectroscopic distinction of signals of the various hemes in a way that is highly sensitive to their redox state.[28,29] This is a consequence of the effect of unpaired electrons from the iron[30,31] on the chemical shift of the signals from nuclei at the periphery of the hemes. In hemes with strong field ligands, the oxidized state is low spin-paramagnetic with the signal of methyl groups of the hemes shifted to the low field region of the NMR spectra, while in the reduced state they appear in the diamagnetic region of the protein (Figure 4). When the intermolecular electron exchange is slow in the time scale of the NMR experiment and intramolecular exchange is fast, a separate set of signals can be observed for the nuclei of the hemes for each oxidation stage (Figure 4). The paramagnetic chemical shift of a signal in intermediate stages is governed by the weighted average of the populations that have that particular heme oxidized in that stage. Therefore, within each column of Figure 1, it is possible to know the population of the states that have each of the hemes oxidized. This allows the fraction of oxidation of each heme to be determined and consequently the ordering of the midpoint reduction potentials of the hemes.[32] Furthermore, the fractions of oxidation of different hemes in different stages are modified by their pairwise interactions, and therefore their effect can be monitored for each stage. Changing the pH changes the balance between protonated and deprotonated states in each stage allowing for the parsing of the electron–proton interactions. Signals belonging to the same nucleus in different stages of oxidation can be identified by exchange spectroscopy NMR experiments. It should be noted that application of this method, which allows the determination of the populations of each state in each stage, is not restricted to heme proteins and was also employed in the analysis of ferredoxins.[33] Since the geometry of the NMR tube makes the measurements of reduction potentials inconvenient due to slow equilibration, the determination of absolute reduction potentials requires complementary data. For the case of heme proteins, redox titrations monitored by UV–visible absorption are an appropriate option. The strong heme absorption bands in UV–visible spectroscopy are also ideal to follow the kinetics of intermolecular electron transfer processes, initiated either by rapid mixing

with redox partners in stopped-flow experiments[34,35] or by generating excited electronic states using laser flashes in flash photolysis.[36]

The thermodynamic and kinetic properties of the redox centers of several multiheme cytochromes are reported in the literature and were used to define the detailed molecular bases for the electron transfer properties of these proteins.[18,35] The most complex example reported is the cytochrome c_3 from *Desulfovibrio desulfuricans* ATCC27774, which contains four hemes and two acid−base centers that are responsible for redox-Bohr interactions in the physiological pH range.[35,37] The largest protein characterized using thermodynamic data derived from NMR and UV−visible spectroscopy experiments was the flavocytochrome c_3 from *Shewanella*, which is an enzyme of 64 kDa.[38] Clearly, as the number of interacting centers increases, the demand on the experimental methods to unequivocally define all necessary parameters increases. This may require new developments in experimental methods before a full microscopic characterization is achieved, as shown for the preliminary characterization of the nonaheme cytochrome from *D. desulfuricans* ATCC27774.[39] As far as heme proteins are concerned, methods that label specific carbon atoms in the hemes of multiheme cytochromes without labeling the remainder of the protein provide enhanced spectral dispersion of the signals in NMR experiments and thus the possibility to discriminate signals in cytochromes with more hemes.[40]

The work reported for the small tetraheme cytochrome from *Shewanella oneidensis* MR-1 will be used to illustrate the power of a microscopic characterization of the individual redox centers to elucidate the detailed mechanism of redox activity of this protein.

The Tetraheme Cytochrome STC from *S. oneidensis* MR-1

The small tetraheme cytochrome (STC) is one of the most abundant soluble proteins found in the periplasm of Gram negative bacteria of the genus *Shewanella* during growth in anaerobic conditions.[41] The X-ray and NMR structural models showed that the hemes in STC are very exposed to the solvent and are arranged as an elongated chain that spans 23 Å for the more distant iron ions. Although the physiological role of this protein is still not settled, it was proposed that it participates in dissimilatory metal reduction, receiving electrons from the inner membrane cytochrome CymA and transferring them to multiprotein complexes that permeate the outer membrane and act as terminal metal reductases at the surface of the cells.[34,42,43]

The redox characterization of STC from *S. oneidensis* MR-1 was attained by combining redox titrations monitored by NMR and UV−visible spectroscopies. It relied on the assignment of the NMR signals to specific hemes in the structure of the protein.[44] The fast intramolecular electron exchange and slow intermolecular electron exchange conditions of the NMR experiments allow these signals to be followed during the redox titrations, providing the necessary data for the oxidation order of the hemes to be elucidated (Figure 4).[44]

These data, together with data obtained through redox titrations followed by UV−visible spectroscopy, were fitted to a thermodynamic model that accounts for the four redox centers and one acid−base center of STC, allowing the individual reduction potential of the hemes, their pairwise interactions, the acid−base pK_a, and the redox-Bohr interactions to be determined.[44] Heme I has the more negative reduction potential, which is followed by the hemes II, IV, and III. Interestingly this order changes to I, II, III, IV above pH 8 by modulation mostly of the potential of heme III.

The thermodynamic properties of STC showed biases in the equilibrium distribution of electrons inside the protein. As a consequence, for each macroscopic stage only one state is significantly populated at physiological pH.

Reduction of STC with sodium dithionite monitored by stopped-flow was performed inside an anaerobic chamber to avoid oxidation by air, and the reducing agent was used in large excess to set pseudo-first-order conditions.[17] The kinetic model provided four reference rate constants for the hemes, which showed that heme I is the sole electron receiver despite having the weakest driving force.[45] Similar experiments were performed for the oxidation of STC with ferric-NTA[18] and ferric-EDTA,[46] showing that heme I is also the major center of electron egress aided by a smaller contribution of heme IV.[18] The importance of these two hemes for oxidation of STC by these iron compounds was independently confirmed by site-directed mutagenesis of aspartates D21 and D80 for asparagines. These residues are located near hemes I and IV, respectively, and the mutants revealed a significant decrease of the oxidation rates.[46]

Only with a detailed microscopic characterization of the redox centers in a multicenter protein is it possible to elucidate the contribution of each redox center for the intermolecular electron transfer process.[45] Given that fast intramolecular electron equilibration within the protein occurs between each of the slower intermolecular electron transfer steps, the thermodynamic order of the reduction potential of the heme responsible for electron uptake or release has consequences for the detailed mechanism of

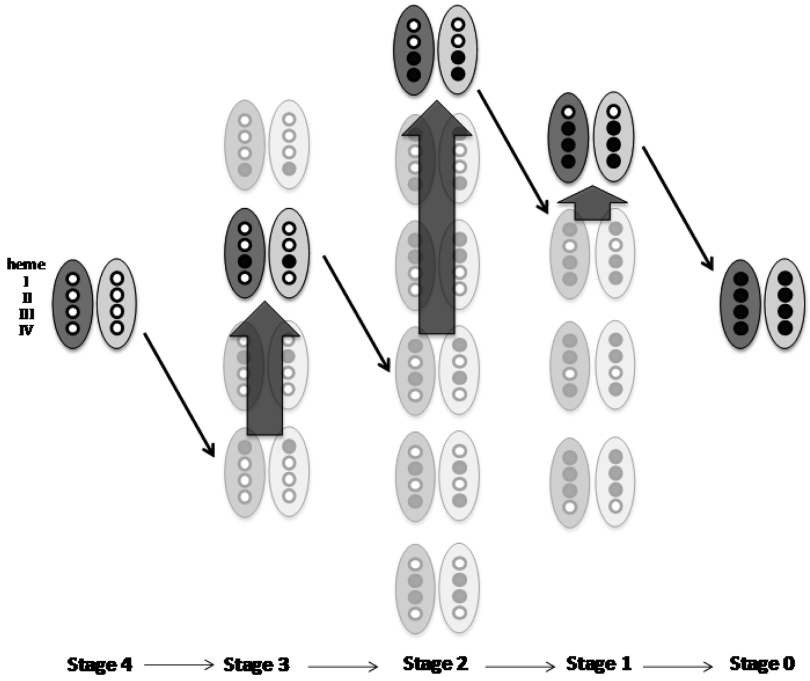

FIGURE 5. Schematic representation of the most populated microscopic states for STC and illustration of intermolecular electron uptake from dithionite (thin arrows) followed by internal reequilibration according to the reduction potentials (broad arrows) that is faster than subsequent electron uptake events.

electron transfer. Figure 5 illustrates how electron uptake from dithionite occurs, with electrons entering through heme I and being distributed in each stage according to the thermodynamic parameters. Interaction with a small partner such as dithionite is not constrained by recognition and docking that usually takes place between physiological redox partners. This appears to be the case for the interaction of STC with its likely physiological electron donor and acceptor, CymA and MtrA. The transient protein–protein complexes were characterized by NMR, and this characterization identified heme IV from STC as responsible for contacting the partners.[34] However, methods that extend the analysis to interpret the multielectron intermolecular electron transfer between these proteins have not been developed yet.

Conclusions

With the report of the structure of complex I of the mitochondrial respiratory chain, all major bioenergetic protein complexes are now characterized from the structural point of view.[47] The scene is now set for determining in detail the molecular mechanisms by which these proteins perform their physiological function. All of them are multicenter redox proteins for which a detailed thermodynamic and kinetic analysis of the electron transfer processes is not a trivial endeavor. However, this information is essential to understand the molecular basis of the intermolecular electron transfer processes that these proteins catalyze, the disruption of which lies at the heart of numerous metabolic disorders. The case study presented here for the small tetraheme cytochrome from *S. oneidensis* MR-1 shows that with a multidisciplinary approach that includes structural, thermodynamic, and kinetic information, it is possible to elucidate the molecular details of intermolecular electron transfer performed by multicenter redox proteins. Notwithstanding our contribution toward these developments has been focused on the study of multiheme cytochromes, the methods presented in this Account are of general application to any multicenter redox protein. They require suitable experimental data that discriminates the redox state of the centers and that the assumptions of slow intermolecular versus fast intramolecular electron transfer are met.

This work was supported by Fundação para a Ciência e a Tecnologia (FCT) – Portugal (Grants PEst-OE/EQB/LA0004/2011, PTDC/BIA-PRO/117523/2010, PTDC/QUI-BIQ/117440/2010, and SFRH/BPD/34591/2007 to C.M.P.) and by COST (Action CM1003: Biological oxidation reactions - mechanisms and design of new catalysts). The authors are grateful to numerous colleagues for stimulating and helpful discussions throughout many years, with particular thanks to Teresa Catarino. We thank Manuela Pereira and Cristina Silva Pereira for critically reading a draft version of the manuscript and the members of the

Inorganic Biochemistry and NMR laboratory for their comments and feedback.

BIOGRAPHICAL INFORMATION

Catarina M. Paquete received her degree in Applied Chemistry from Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa (1999). In 2006, she received her Ph.D. in Biochemistry from Instituto de Tecnologia Química e Biológica of Universidade Nova de Lisboa. Since 2007, she is developing her Postdoctoral work in the same Institute and in collaboration with Prof. Ming Tien at Penn State University to develop molecular biology strategies that allow an in-depth characterization of multiheme cytochromes.

Ricardo O. Louro received his degree in Applied Chemistry from Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa (1993). In 1998, he received his Ph.D. in Biochemistry from Instituto de Tecnologia Química e Biológica of Universidade Nova de Lisboa for work on the functional consequences of electron–proton coupling in multicenter redox proteins. His research interests have been focused on the interface between inorganic chemistry and bioenergetics, which he explores using paramagnetic NMR methods for the functional characterization of redox proteins and enzymes.

FOOTNOTES

*Corresponding author. Phone: +351 21 4469309. Fax: +351 21 4411277. E-mail: louro@itqb.unl.pt.

The authors declare no competing financial interest.

The manuscript was written through contributions of both authors. All authors have given approval to the final version of the manuscript.

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