PROTEIN DYNAMICS, POTENTIAL REGULATION, AND REDOX COUPLED CONFORMATIONAL CHANGES IN CYTOCHROMES *c*

F. R. Salemme[1]

Department of Biochemistry
University of Arizona
Tucson, Arizona

I. INTRODUCTION

The term "conformational change" is generally applied to situations in which the initial and final static configurations of a molecule differ, usually as a result of the molecule binding some ligand or undergoing some chemical alteration. A well known example is the conformational change induced in the hemoglobin tetramer upon the binding of oxygen. However, protein molecules are not static structures, but are constantly undergoing thermally induced vibrations. For at least some molecules which appear to undergo "conformational" changes, it appears that these changes do not reflect static configurational alterations, but instead manifest an alteration in the dynamical state of the protein. Such changes in the dynamical state of a protein are necessarily accompanied by energetic changes in the system, and therefore are of some interest with regard to the more general problems of energy transduction in proteins. Indeed, recent investigations suggest that proteins may have coherent vibrational modes which involve large parts of the molecule.

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II. DYNAMICS OF CYTOCHROMES c

The cytochromes c constitute one of the most comprehensively studied classes of proteins (1-4). Members of the class of molecules show an extremely wide species diversification; indeed, they appear to play a functional role in virtually all living organisms which utilize electron transport chains as a primary means for synthesizing ATP. Generally, the soluble cytochromes c serve as specific isopotential electron carriers, catalyzing the sequential oxidation and reduction of associated membrane-bound oxidoreductases.

As a class, the soluble cytochromes c are characterized by their relatively small size (85 to 135 amino acid residues), and the presense of a covalently bound heme IX prosthetic group, whose iron atom is axially ligated by a histidine imadazole nitrogen and a methionine sulfur atom furnished by the polypeptide backbone. These latter strong-field axial ligands confer low-spin character upon the heme iron in both its singly charged oxidized and uncharged reduced state.

The structures of several species of cytochrome c have been determined by crystallographic techniques and show the existence of considerable structural similarity among species as diverse as horse (5) and the green photosynthetic sulfur-bacterium Chlorobium thiosulfatophilum (6) (Fig. 1). Comparative biochemical and structural studies have been useful in defining the factors which confer specificity upon the reactivity of cytochrome c, and have given insight into the possible nature of the electron transfer mechanism (7). Nevertheless, there are important physicochemical properties of the molecules which manifest themselves in solution, and which might reasonably be expected to have structural correlates, about which the time-averaged X-ray structure provides little information. These include 1) the oxidoreduction coupled "conformational" change undergone by soluble cytochromes c, and 2) the origin of the differences in observed midpoint potential for the identical protoheme IX prosthetic groups in different cytochrome c species.

There exists a wide variety of solution data which indicate that the oxidoreduction of cytochrome c is accompanied by a conformational change. These

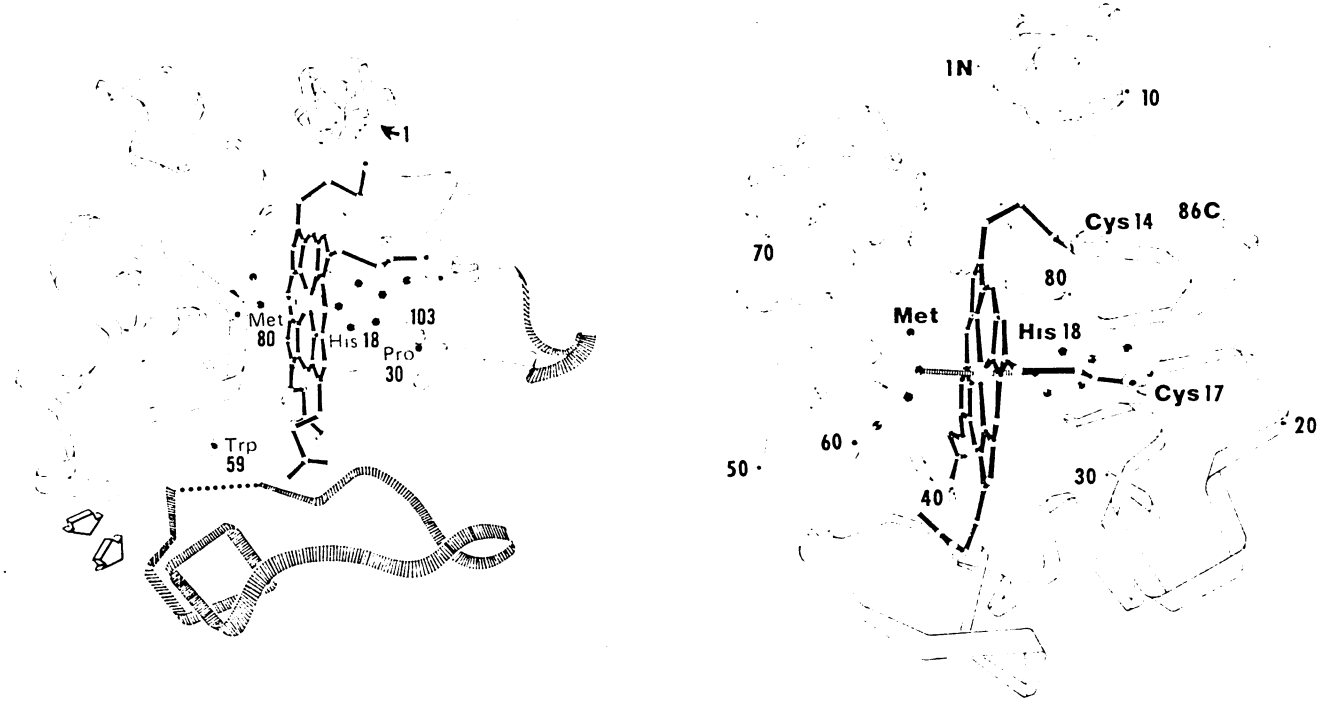

Figure 1. Schematic representations of eucaryotic mitochondrial cytochrome c (left) and cytochrome c555 from the photosynthetic green sulfur bacterium Chl. thiosulfatophilum. The bacterial cytochrome structurally differs from the eucaryotic cytochrome c by a series of structural deletions which are shown shaded in the view at the left. Note that the structural deletions in the bacterial molecule are accompanied by a rearrangement of the polypeptide chain so that the hydrophobic heme environment is preserved in the smaller molecule.

include increased susceptibility of the oxidized molecule to proteolytic digestion (8), increased sensitivity to solvent induced or thermal denaturation (9-13), increased accessibility of the heme iron to exogenous ligands in the oxidized state (15-17), and increased rate and extent of the deuterium exchange in the oxidized molecular form (18, 19). All of these observations suggest that the oxidized form of the molecule possesses a more open and/or more loosely organized structure than the reduced form. However, crystallographic structure determination of the reduced and oxidized forms of the molecule show the conformations of the alternate redox states to be essentially identical (20-23). A reasonable explanation which reconciles these seemingly contradictory results is that the difference between the oxidized and reduced molecular forms reflect a change in their dynamic vibrational properties. For example, Figure 2 shows generalized classical harmonic oscillator potentials for the oxidized and reduced cytochrome c molecules. The potential for the reduced molecule is shown steeper than for the oxidized form, consistent with the greater apparent rigidity of the reduced structure. At physiological temperatures, the reduced form will oscillate about its equilibrium configuration (E_o). The magnitude of these oscillations will be larger in the oxidized form owing to the "softer" character of its oscillator potential. These larger dynamic excursions of the oxidized molecule account for its solution properties. However, this difference in the dynamical states of the two forms is difficult to detect in the time-averaged X-ray structures, both since there appears to be little difference between the equilibrium configurations of the two oxidation states, and the X-ray experiment is subject to additional types of disorder which obscure the effects of molecular motion. The physical event which governs the interconversion between these two vibrational states is the reduction of the heme iron which is known to be accompanied by a large increase in the strength of the heme iron-axial ligand methionine sulfur bond (24). As can be seen from Figure 1, this bond forms one of the few covalent links which hold the left and right sides of the cytochrome c molecules together.

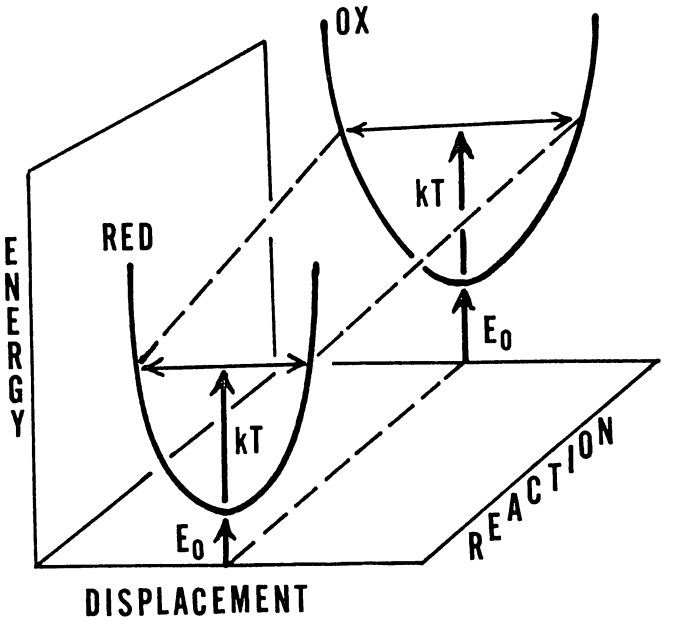

Figure 2. A schematic diagram illustrating the possible vibrational motions of the oxidized and reduced cytochrome c structures. Note that the energy difference between the oxidized and reduced states is dependent in part upon how much ambient thermal energy can partition into the different vibrational states of the two molecular forms, in addition to any energy difference which might exist between the E_0 states.

To summarize, it appears that the "conformational" change accompanying a redox state change in cytochrome c is most readily explained by a change in the accessible dynamic states of the molecule, rather than by some alteration in the equilibrium configuration which would presumably manifest itself as a static structural difference between the two molecular forms.

The structural factors responsible for the differences in observed midpoint potential for various cytochromes remain obscure. Although arguments made by Kassner (25, 26) relating oxidoreduction potential to heme hydrophobicity may account for the generally high potentials of cytochromes c relative to aqueous heme complexes, recent structural studies of C. thiosulfatophilum c555 indicate that this low potential ($E_{m,7}$ = +145mV) cytochrome does not significantly differ in the extent of its heme exposure from high potential cytochromes c (e.g. R. rubrum

cytochrome c_2, $E_{m,7}$ = +320mV). This result suggests that subtle adjustments in redox potential may be achieved by alteration of some as yet unrecognized localized protein-heme interaction. Alternatively, it is possible that small differences in redox potential in proteins with otherwise identical prosthetic groups may result from relative differences in the vibrational properties of the molecules in either of their oxidation states. This follows since the protein oxidoreduction potential reflects the difference in free energy between the oxidized and reduced states, which appears to manifest itself as a change in the accessible dynamical states of the molecule. These could differ in proteins having relatively small differences in their structures.

III. COHERENT DYNAMIC PROCESSES IN PROTEINS AND ENERGY CONSERVATION

The basic point of the preceeding discussion of the oxidoreduction properties of cytochrome c, is that the free energy change in the molecule accompanying oxidoreduction manifests itself as a change in the dynamic properties of the molecule as a whole despite the fact that the change in dynamic state appears to be triggered by a localized chemical event, heme oxidoreduction. This raises an interesting possibility for mechanisms of transient energy storage in non-isopotential, energy-transducing oxidoreduction proteins; i.e. energy is transiently stored as an excited molecular vibrational state. At present, it is not clear whether proteins have a sufficient vibrational energy bandwidth to accommodate the free energy change associated with proton translocation or ATP formation. Nevertheless, recent investigations of the motion of regular secondary structures of proteins (Weatherford and Salemme, in press) indicate that much of the vibrational motion of proteins may be coherently coupled. For example, it appears that the fundamental vibrational mode of an extended β-sheet structure approximates that of a torsional oscillator. It is possible that the coherent vibrations of such high mass systems may be of sufficient energy to play a role in energy transduction. More importantly, however, these effects necessarily propagate throughout the

structures over very long distances (i.e. tens of Ångstroms), and thus potentially overcome one of the difficult conceptual problems associated with energy coupling, the remoteness of the site of non-isopotential electron transfer from the site of proton translocation or ATP synthesis.

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