An Hypothetical Structure for an Intermolecular Electron Transfer Complex of Cytochromes c and b_5

F. R. SALEMME

Department of Chemistry, University of Arizona
Tucson, Ariz. 85721, U.S.A.

An hypothetical structural complex of cytochromes c and b_5 which optimizes intermolecular complementary charge and steric interactions was generated by a least-squares fitting process. The interactions formed in this complex constrain the two heme prosthetic groups to be nearly coplanar at a closest approach distance for resonant heterocyclic porphyrin ring atoms of 8·4 Å. The structural properties of the complex are discussed in the general context of oxidoreduction processes carried out by reversibly bound protein electron carriers.

1. Introduction

The mechanism by which electrons are transferred to or from soluble electron carrier proteins during interactions with physiological oxidoreductases remains obscure despite the availability of several high resolution X-ray structures and a considerable quantity of related biochemical data. It has recently been suggested that the electron transfers mediated by c-type cytochromes take place by mechanisms involving essentially direct interactions between the c- type cytochrome heme prosthetic group and the prosthetic groups of the corresponding oxidoreductases (Salemme *et al.*, 1973a). The experiment described here was carried out to test the structural reasonability of this proposal by finding the best mechanical fit between two heme proteins of known structure and reactivity; tuna heart mitochrondrial cytochrome c (Takano *et al.*, 1973) whose structure was determined by R. E. Dickerson and co-workers at California Institute of Technology (who kindly furnished the atomic co-ordinates), and microsomal cytochrome b_5 (Mathews *et al.*, 1972) as determined by F. S. Mathews, P. Argos and M. Levine at Washington University at St. Louis (co-ordinates from the Protein Data Bank, Brookhaven National Laboratories).

The selection of mitochondrial cytochrome c and microsomal cytochrome b_5 for this study stemmed from a variety of structural and biochemical observations.

(1) All species of c-type cytochromes of currently known structure show the presence of a sequentially and/or structurally conservative group of lysine residues which form an uninterrupted ring of positive charge at the front of the molecule, where the otherwise buried heme is most exposed to solvent (Fig. 1(a)). A diversity of experimental approaches has established the functional importance of this belt of positive charge in mediating the interactions of c-type cytochromes with physiological oxidoreductases. Overall, these data are consistent with the proposal that electron transfer is facilitated by a complementary ionic interaction between the c-type cytochromes and their respective oxidoreductases (Salemme *et al.*, 1973a).
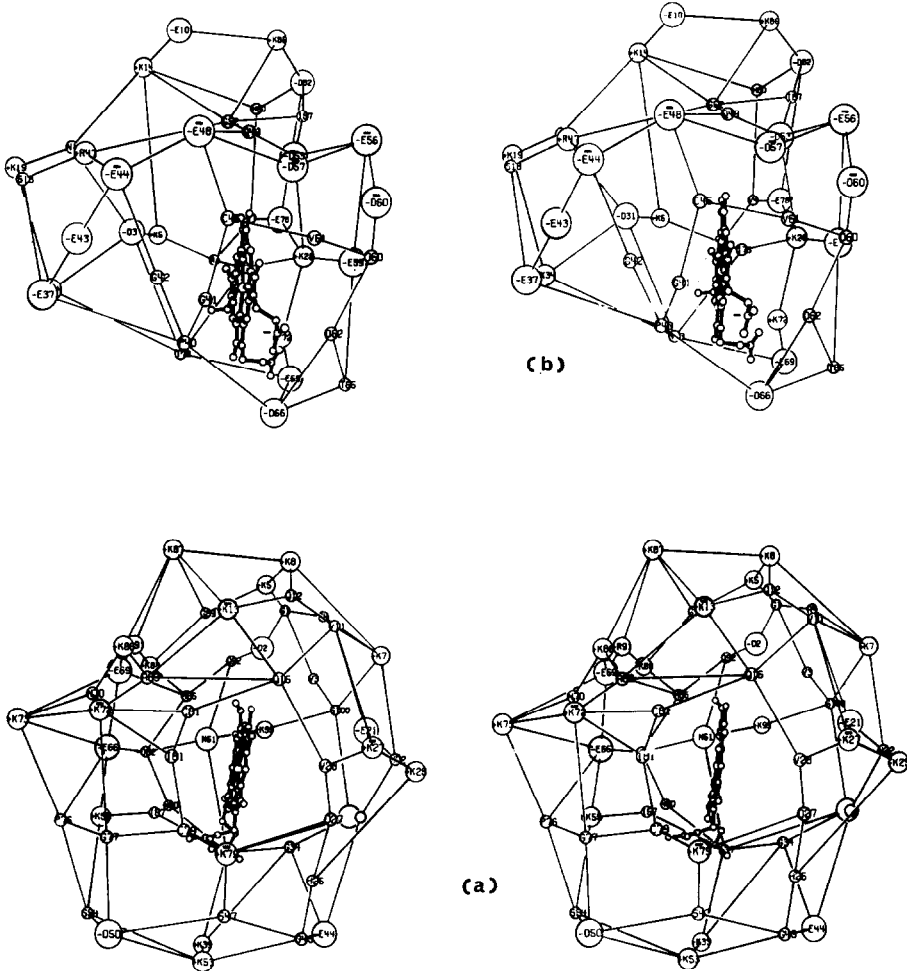
Fig. 1. Polyhedral surface maps of the cytochrome c (a) and cytochrome b_5 (b) molecules. Surface residues were defined by the position of the most exposed atom, except in the case of acidic side-chains which were defined by carboxylic carbon atom position, and are indicated by charge, residue and sequence number. Additional atoms not conforming to this labeling pattern are shown to define better the local surface topography of the respective heme crevice perimeters. These are for cytochrome c: O27 (Lys27 peptide oxygen), C79 (Lys79 Cγ), C81 (Ile81 Cγ1); for cytochrome b_5: C45 (Ile45 Cγ2), O60 (Asp60 peptide oxygen), O62 (Gly62 peptide oxygen). A heavy bar denotes essential charge invariance in all species of cytochrome b_5 and some 50 species of eucaryotic cytochrome c. Single letter code: D = Asp, E = Glu, K = Lys, R = Arg, H = His, A = Ala, P = Pro, G = Gly, S = Ser, T = Thr, W = Trp, F = Phe, I = Ile, L = Leu, M = Met, N = Asn, Q = Gln, V = Val, Y = Tyr, C = Cys.

(2) Microsomal cytochrome b_5 exhibits similar clustering of charged residues about the surface region where the heme is most accessible to solvent, except that in contrast to the situation for c-type cytochromes the charge belt is composed of negatively charged groups contributed by surface glutamic or aspartic acid residues and one exposed heme propionic acid side chain (Fig. 1(b)). Significantly, the majority of these surface residues are charge-conserved in all species of microsomal cytochrome b_5 sequenced to date (Dayhoff, 1972). Cytochrome b_5 is known to reduce cytochrome c efficiently *in vitro* (Strittmatter, 1964) at rates comparable to those observed for

the physiological oxidoreductions of both molecules in their native environments. The observed rates of oxidoreduction of cytochrome b_5 with protein oxidoreductases are generally dependent upon ionic strength, again suggesting that the association of this molecule with protein oxidoreductases is mediated by complementary charge interactions (Passon & Hultquist, 1972). The question whether cytochrome c functions as a physiological oxidant of cytochrome b_5 remains controversial in the absence of definitive evidence. However, the presence of a b_5-type cytochrome anchored to the inner surface of the outer mitochondrial membrane, and hence capable of direct interaction with mitochondrial cytochrome c localized in the intramitochondrial space, suggests that cytochrome c may serve as a minor physiological oxidant of cytochrome b_5.

Taken together, the preceding observations suggested that an investigation of the potential structural complementarity of the cytochrome c and b_5 molecules would at the least define the minimum approach distance between the prosthetic groups of protein molecules known to react readily with each other. The possibility that the cytochrome c–cytochrome b_5 interaction might be physiologically significant, or at least structurally typical of such interactions, provided an additional basis for the selection of molecules in this experiment.

2. Materials and Methods

(a) Generation of the hypothetical complex structure

Stereo drawings of the surface topographies of the cytochrome c and b_5 molecules were generated by connection of the most exposed atoms of surface amino acid residues to form irregular polyhedra. Surface atoms were defined for each molecule by reference to static solvent accessibility calculations (Shrake & Rupley, 1973) which were carried out by J. Rupley. Examination of the resulting surface maps for regions of charge clustering verified previous observations that the front side (i.e. facing the heme crevice) of both molecules showed the most extended regions of similar charge (Fig. 1). Consequently, subsequent attempts to generate the best hypothetical structural complex between the 2 molecules focussed upon possible front-to-front intermolecular interactions.

Initially, the principal criterion used in evaluating the relative likelihood of complex formation between the cytochrome c and b_5 molecules was the quality of complementary fit between the sequentially invariant positive charge groups about the heme crevice in cytochrome c and the invariant negative charge groups about the heme crevice in cytochrome b_5. Preliminary fitting was carried out by calculating a least-squares plane through the frontside charge invariant groups of each molecule, and subsequently plotting the charge group positions relative to their respective least-square reference planes. This procedure provided topological surface charge maps in comparable reference frames and facilitated recognition of potential complementary interactions. For the invariant surface residues of cytochrome c (Lys13, Lys27, Lys72, Lys79, defined by terminal nitrogen atom position) and cytochrome b_5 (heme propionate, Glu44, Glu48, Glu56, Asp57, Glu59, Asp60, defined by carboxylic carbon atom position) it appeared that for both molecules, the charged residues lay on roughly complementary saddle-shaped surfaces. This result suggested 2 possible complementary fits for the 2 molecules in a hypothetical intermolecular complex, differing by a rotation of approx. 180° about an axis normal to the center of the saddle surfaces. Atomic co-ordinates for these 2 intermolecular complexes were generated by least-squares rotation of the appropriate cytochrome b_5 carboxylic carbon atom positions onto points extended 4·5Å from the cytochrome c lysine terminal nitrogen atom positions in a direction normal to their defining least-squares plane. This extension procedure was designed to give a final interatomic distance of approximately 3Å between the lysine terminal nitrogen atoms of cytochrome c and the carboxylate oxygen atoms of the rotated cytochrome b_5 molecule in the resulting intermolecular complexes.

37

Both of the resultant complexes gave satisfactory complementary fits between the surface charge groups of the two molecules. However, detailed examination of all potential intermolecular contacts in both complexes revealed that a significant fraction of prohibitively close contacts between polypeptide backbone atoms, C_β carbon atoms, or otherwise structurally constrained side chain atoms existed, along with some "holes" at the interface regions. Consequently, further checks of the structural complementarity in these complexes were made using rigid space-filling models. From the model work it was apparent that intimate fits optimizing both ionic and non-ionic intermolecular interactions, which closely resembled the initial fits based upon charge complementarity alone, could be obtained if some rotation of the cytochrome c lysine side chains about their C_β—C_γ bonds was allowed. This approach appeared reasonable since lysine residues are generally solvent exposed beyond their C_β side-chain carbon atoms and, indeed, usually appear disordered in protein crystallographic electron density maps. Subsequent least-squares fitting attempts consequently incorporated several additional non-ionic surface atoms of both molecules to define their surface topographies more exactly, and to weight the least-squares fitting process so that overall, both ionic and non-ionic intermolecular interactions were optimized. At this stage it became apparent that one of the initial orientations, when adjusted in the above manner, gave a significantly better complex in terms of surface structural complementarity than the other. It is this "best" structural complex that is discussed below.

3. Results

The complex representing the best obtained intermolecular fit between the cytochrome c and b_5 molecules is shown in Figure 2. There are four principal complementary charge interactions formed in the complex giving a lysine terminal nitrogen–carboxylic oxygen separation of approximately 3Å, assuming rotation of lysine side chains about the C_β—C_γ bond. These interactions are formed between the cytochrome c lysine residues at positions 13, 27, 72, 79 and the cytochrome b_5 carboxyl groups of Asp48, Glu44, Asp60, and the most exposed heme propionate, respectively. Examination of the intermolecular contacts between polar and aliphatic amino acid side chains in the interface region, whose perimeter is defined by the charge interactions given above, indicates that bulk water would be effectively excluded from the majority of this region during complex formation. The overall pattern of both the non-ionic steric interactions and complementary charge interactions formed at the complex interface restrictively orients the heme prosthetic groups of the molecules in nearly the same plane, the dihedral angle formed between the two heme planes being approximately 15°. The closest interatomic distance of approach between heterocyclic Π-bonded atoms of the two heme prosthetic groups of the complex is 8·4 Å.

4. Discussion

Although it is not possible to evaluate rigorously the goodness of complementary fit between the cytochrome c and b_5 molecules in this hypothetical complex in terms of the likelihood that it represents a functional physiological entity, it can be seen that, overall, the intermolecular fit is quite good (Fig. 2). It is most notable that the pattern of interactions formed at the complex interface serves to restrict the heme prosthetic groups of the two molecules to nearly coplanar orientations, a situation which would be expected to facilitate electron transfer between them by some direct mechanism. Although the closest interatomic distance of approach between Π-bonded atoms of the resonant heme heterocycles appears somewhat long
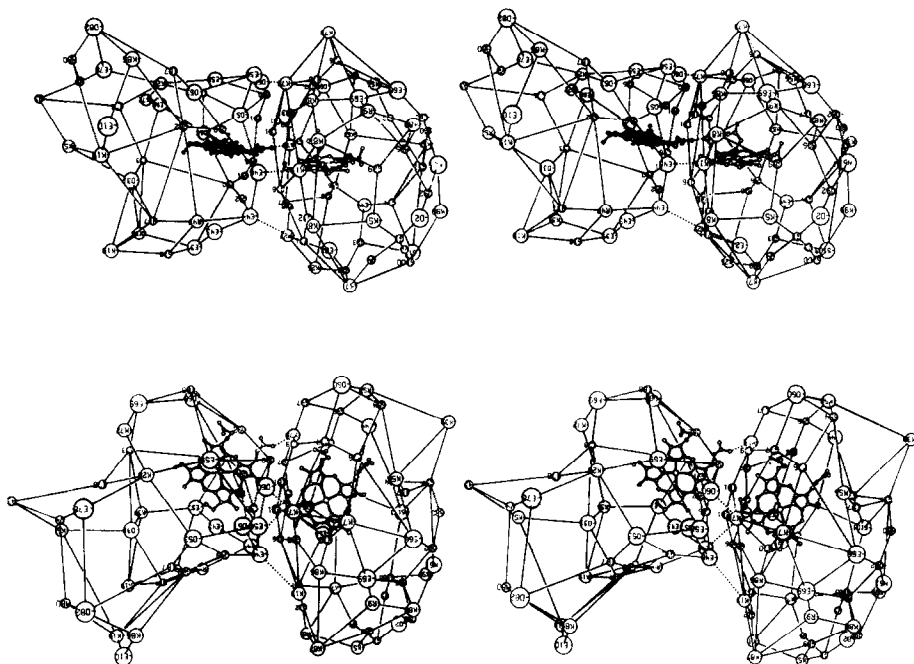
Fig. 2. Polyhedral surface maps of the intermolecular complex of cytochromes c and b_5, showing top and side views. Cytochrome c is on the left of the complex. Dotted lines show principal charge interactions from charge group positions defined by original co-ordinate sets. The best fit shown was obtained allowing free rotation of lysine side chains of cytochrome c about the C_β—C_γ side-chain bond.

at 8·4 Å, in the absence of information concerning the extent of delocalization of either the donor or acceptor orbitals of the prosthetic groups it is difficult to speculate whether this suggests electron transfer by a classical outer sphere mechanism or by a short-range tunnelling mechanism (Hopfield, 1974). In either case, it is relevant to note that considerable delocalization of unpaired spin density in ferricytochrome c has been observed by nuclear magnetic resonance methods (Redfield & Gupta, 1971), and that this spin density appears localized in a region which would coincide with the shortest path connecting the two heme iron atoms of the complex (Salemme et al., 1973b). Additional facilitation of the electron transfer process might arise due to both the close association of polar and hydrophobic side chains in the complex inter-face region and the complementary neutralization of many of the charge groups immediately peripheral to the heme crevices of both molecules. Both the hydrophobic association resulting in the exclusion of water at the interface and the complementary charge neutralization would tend to lower the dielectric constant greatly along the shortest path of interheme electron transfer, consequently deshielding the hemes relative to their situation in the isolated molecules.

Insofar as a strong dependence of oxidoreduction rate on ionic strength has been found to be a general property of reactions of reversibly bound electron carriers with their physiological oxidoreductases, it is appropriate to comment upon the possible origins of the forces which might result in complex formation of the type described here. The relevant structural observation is that the interacting charge groups in the

cytochrome $c–b_5$ complex are members of larger surface charge domains on the respective molecular surfaces (Fig. 1) which are of *like* charge. Since the tertiary conformation of these molecules constrains these like-charged groups in close proximity to each other, the formation of the complex can be likened to the crystallization of an ionic solute. In the latter case, when the solute concentration becomes sufficiently high, so that there is not sufficient water to allow all solute ions to be optimally solvated, the solute is forced to make an alternative set of stabilizing ionic interactions and it crystallizes. In like manner, the presence of many groups of like charge clustered in close proximity on the surface of a protein, as is the case for the cytochrome c and b_5 molecules, would be expected to create a situation in which ionic bond formation could compete with the usual water hydration of such groups. Indeed, as shown in Figure 1, the like charge nature of the groups proximal to the heme crevice in both cytochromes c and b_5, appears to be evolutionarily conserved as would be expected in light of their proposed functional role.

REFERENCES

Dayhoff, M. O. (1972). In *Atlas of Protein Sequence and Structure*, p. D-30, National Biomedical Research Foundation, Washington, D.C.

Hopfield, J. J. (1974). *Proc. Nat. Acad. Sci., U.S.A.* **71**, 3640–3644.

Mathews, F. S., Argos, P. & Levine, M. (1972). *Cold Spring Harbor Symp. Quant. Biol.* **36**, 387–395.

Passon, P. G. & Hultquist, D. E. (1972). *Biochim. Biophys. Acta*, **275**, 62–73.

Redfield, A. G. & Gupta, R. K. (1971). *Cold Spring Harbor Symp. Quant. Biol.* **35**, 405–412.

Salemme, F. R., Kraut, J. & Kamen, M. D. (1973a). *J. Biol. Chem.* **248**, 7701–7716.

Salemme, F. R., Freer, S. T., Xuong, N. H., Alden, R. A. & Kraut, J. (1973b). *J. Biol. Chem.* **248**, 3910–3921.

Shrake, A. & Rupley, J. A. (1973). *J. Mol. Biol.* **79**, 351–371.

Strittmatter, P. (1964). In *Rapid Mixing and Sampling Techniques in Biochemistry* (Chance, B., Eisenhardt, R. H., Gibson, Q. H. & Lunberg-Holm, K. K., eds), pp. 71–84, Academic Press, New York.

Takano, T., Kallai, O. B., Swanson, R. & Dickerson, R. E. (1973). *J. Biol. Chem.* **248**, 5234–5246.