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Cooperative ligand reorientations in cytochrome c_3: a molecular dynamics simulation

P.C. Weber, J.J. Wendoloski and F.R. Salemme *

Central Research and Development Department, E.I. DuPont de Nemours & Co., Inc., Wilmington, DE (U.S.A.)

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Molecular dynamics simulations of a tetraheme cytochrome c_3 were performed to investigate dynamic aspects of the motion of the axial heme iron ligands. It was found that persistent transitions between alternate axial imidazole orientations of the histidine incorporated in the CXXCH heme binding sequence occurred via correlated motions. The correlated motions involved virtually all of the atoms comprising the polypeptide backbone of the heme binding sequence as well as the histidine imidazole side-chain.

Introduction

Heme-containing proteins perform a wide variety of catalytic and electron transfer functions in biological systems. For some years we have studied the structural architecture [1–4] and mechanistic basis for redox potential regulation [2,5] and electron transfer specificity [6–8] between biological redox proteins that incorporate heme prosthetic groups. Interaction of the heme prosthetic group with the surrounding protein environment causes it to manifest a wide range of alternative physical states reflected in the heme group's electronic, magnetic spin state, electromotive potential and oxidation state. Previous work used results obtained from high-resolution crystallographic studies of proteins to examine the role of electrostatic interactions in facilitating and conferring specificity upon intermolecular electron transfer reactions [2,6,7]. More recently, detailed molecular dynamics simulations were performed on a complex of two heme proteins, cytochrome *c* and cytochrome b_5 [8]. The simulations illustrated the role of conformational sampling in providing configurations that could effectively couple heme electronic systems and facilitate electron transfer. This work has now been extended to several additional systems, including the dimeric protein cytochrome *c'* [4] and the tetra-heme cytochrome c_3 [9], which is described here. In the case of cytochrome c_3, we focused on an analysis of possible concerted motions of the histidine residues that axially coordinate the heme iron atoms. The study was motivated by a structural observation that the coordinating histidine iron ligand and associated covalent heme binding sequences, which generally include the sequence cysteine-X-X-cysteine-histidine (CXXCH) that forms thioether linkages to the heme as well as furnishing an iron imidazole ligand, were oriented in distinctly different conformational substates in different cytochrome *c* structures [4]. Molecular dynamics simulations were carried out to examine the possibility of transient or persistent transitions between these conformational subtypes that could reflect dynamic alterations of heme physical properties relevant to electron transfer or catalysis.

Methods

Cytochrome c_3 is a protein of 107 residues containing four covalently bound, heme prosthetic groups. Two similar sequences covalently bind the heme groups of the molecule. Two of the heme groups are attached by CXXCH sequences, where X designates any amino acid, and two by CXXXXCH sequences. The former sequence is most common among *c*-type cytochromes and displays two distinct conformations in this molecular family [4]. The structure of *Desulfovibrio vulgaris* cytochrome c_3 has been refined to a crystallographic *R*-factor of 0.176 at 1.8 Å resolution [9], and coordinates for the dynamics simulations were obtained from the Brookhaven Protein Data Bank [10]. The relatively short

* Present address: Chemical Research & Development, Sterling Research Group, Malvern, PA, U.S.A.

Correspondence: P.C. Weber, The DuPont Merck Pharmaceutical Company, DuPont Experimental Station E228/320, Wilmington, DE 19880-0228, U.S.A.

polypeptide chain of cytochrome c_3 contains few regions of regular secondary structure. Ten reverse turns direct the polypeptide to fold about the four heme prosthetic groups. Cysteine residues 100 and 105, 46 and 51, 30 and 33, and 79 and 82 form thioether linkages with the vinyl groups of hemes 1 through 4. Histidine residues 70 and 106, 35 and 52, 22 and 34, and 25 and 83 provide nitrogen axial ligands to heme groups 1 through 4.

Molecular dynamics simulations were carried out on a STAR ST100 array processor using the program AMBER [11] with a united atom force field. The X-ray structure, including crystallographically observed water molecules, was minimized using 1000 steps of steepest descent minimization followed by 100 cycles of conjugate gradient minimization. The system was heated during an initial 5 ps of simulation, followed by 20 ps of equilibration, and an additional 100 ps of simulation providing a trajectory for analysis. Simulations were run using a time step of 1 femtosecond (fs) with coupling to a thermal bath at 300 K using the method of Berendsen et al. [12] with a temperature relaxation constant of 0.1 ps. Bond lengths involving hydrogens were held fixed using the SHAKE algorithm [13] and a distant dependent dielectric constant of $e_{ps} = R_{ij}$ with a 9.5 Å nonbonded cutoff was used for nonbonded interactions.

Results and Discussion

Each heme iron in cytochrome c_3 forms axial ligands to histidine imidazole groups through Nϵ2. Structural comparisons of *c*-type cytochromes [4] show that two ligand orientations occur for the imidazole rings in the CXXCH sequences, which differ by an approx. 90° rotation about the imidazole Nϵ2-Heme Fe bond, and correspond to alternative staggered orientations of the imadazole group relative to the Fe-pyrrole nitrogen bonds of the heme. The dynamic simulations show average r.m.s. fluctuations in heme-imidazole dihedral angle of 10° to 15° (with most excursions remaining in a quadrant bounded by eclipsed imidazole orientations) and little apparent difference in behavior between imidazoles in CXXCH or CXXXXCH linkages and the remaining histidine iron ligands. However, more detailed inspection occasionally shows larger transient excursions of as much as 85°. While some of these are only locally correlated, generally occur within a quadrant between staggered conformation, and are transient in nature, others represent transitions from staggered conformations over an eclipsed 'barrier'. Most interestingly, persistent excursions, where the imidazole group rotates from one staggered orientation to another, involve correlated movement of a number of polypeptide backbone atoms in the CXXCH loop in a U-shaped trajectory. Consequently, the alternative staggered conformations seen in the X-ray crystal structure of *c*-type cytochromes appear to represent alternative conformational minima of the CXXCH structural unit which the dynamics simulations show can be interconverted via a correlated conformational trajectory. Although more theoretical and experimental studies are needed to demonstrate the functional role of such correlated motions in heme protein function, the motion observed is anticipated in the work of Ansari et al. [14], who suggested the importance of correlated FIMs, or functionally important motions, in the ligand binding kinetics of globins.

References

1 Salemme, F.R., Kraut, J. and Kamen, M.D. (1973) J. Biol. Chem. 248, 7701–7716.
2 Salemme, F.R. (1977) Annu. Rev. Biochem. 46,229–329.
3 Korszun Z.R. and Salemme, F.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5244–5247.
4 Finzel, B.C., Weber, P.C., Hardman, K.D. and Salemme, F.R. (1985) J. Mol. Biol. 186, 627–643.
5 Salemme F.R. (1978) in Frontiers of Biological Energetics (Dutton, P.L, Leigh, S. and Scarpa, A., eds.), pp. 83–89, Academic Press, New York.
6 Salemme, F.R. J. Mol. Biol. (1976) 102, 563–568.
7 Matthew, J.J., Weber, P.C., Salemme, F.R. and Richards, F.M. (1983) Nature 301, 169–171.
8 Wendoloski, J.J., Matthew, J.J., Weber, P.C. and Salemme, F.R. (1987) Science 238, 794–796.
9 Higuchi, Y., Kusunoki, M., Matsurra, Y., Yasuoka, N. and Kakudo, M. (1984) J. Mol. Biol. 172, 109–139.
10 Bernstein, F.C., Koetzle, T.F., Williams, G.J.B., Meyer, E.F. Jr., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, M. (1977) J. Mol. Biol. 112, 535–542.
11 Weiner, S.J., Kollman, P.A., Case, D.A., Singh, U.C., Ghio, C., Alagona, G. and Weiner, P. (1984) J. Am. Chem. Soc. 106, 765–784.
12 Berendsen, H.J.C., Postma, J.P.M., DiNola, A., Van Gunsteren, W.F. and Haak, J.R. (1984) J. Chem. Phys. 81, 3684–3690.
13 Van Gunsteren, W.F. and Berendsen, H.J.C. (1977) Mol. Phys. 34, 1311–1327.
14 Ansari, A., Berendzen, J., Bowne, S.F., Frauenfelder, H., Iben, I.E.T., Sauke, T.B., Shyamsunder, E., Young, R.D. (1985) Proc. Natl. Acad. Sci. USA, 82, 5000–5004.