doi:10.1016/j.jmb.2011.09.023 J. Mol. Biol. (2011) 414, 145–162

Contents lists available at www.sciencedirect.com

Journal of Molecular Biology

journal homepage: http://ees.elsevier.com.jmb

Conservation of Lipid Functions in Cytochrome *bc* Complexes

S. Saif Hasan [1], Eiki Yamashita [2], Christopher M. Ryan [3], Julian P. Whitelegge [3] and William A. Cramer [1]*

[1]*Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA*
[2]*Institute of Protein Research, Osaka University, Osaka 565-0871, Japan*
[3]*Pasarow Mass Spectrometry Laboratory, Neuropsychiatric Institute–Semel Institute, University of California, Los Angeles, CA 90024, USA*

Received 25 May 2011;
received in revised form
5 September 2011;
accepted 14 September 2011
Available online
28 September 2011

Edited by R. Huber

Keywords:
structure;
membrane proteins;
lipids;
cytochrome b6f;
cytochrome bc1

Lipid binding sites and properties are compared in two sub-families of hetero-oligomeric membrane protein complexes known to have similar functions in order to gain further understanding of the role of lipid in the function, dynamics, and assembly of these complexes. Using the crystal structure information for both complexes, we compared the lipid binding properties of the cytochrome b_6f and bc_1 complexes that function in photosynthetic and respiratory membrane energy transduction. Comparison of lipid and detergent binding sites in the b_6f complex with those in bc_1 shows significant conservation of lipid positions. Seven lipid binding sites in the cyanobacterial b_6f complex overlap three natural sites in the *Chlamydomonas reinhardtii* algal complex and four sites in the yeast mitochondrial bc_1 complex. The specific identity of lipids is different in b_6f and bc_1 complexes: b_6f contains sulfoquinovosyldiacylglycerol, phosphatidylglycerol, phosphatidylcholine, monogalactosyldiacylglycerol, and digalactosyldiacylglycerol, whereas cardiolipin, phosphatidylethanolamine, and phosphatidic acid are present in the yeast bc_1 complex. The lipidic chlorophyll *a* and β-carotene (β-car) in cyanobacterial b_6f, as well as eicosane in *C. reinhardtii*, are unique to the b_6f complex. Inferences of lipid binding sites and functions were supported by sequence, interatomic distance, and *B*-factor information on interacting lipid groups and coordinating amino acid residues. The lipid functions inferred in the b_6f complex are as follows: (i) substitution of a transmembrane helix by a lipid and chlorin ring, (ii) lipid and β-car connection of peripheral and core domains, (iii) stabilization of the iron–sulfur protein transmembrane helix, (iv) n-side charge and polarity compensation, and (v) β-car-mediated super-complex with the photosystem I complex.

**Corresponding author*. E-mail address: waclab@purdue.edu.

Abbreviations used: β-car, β-carotene; Chl *a*, chlorophyll *a*; cyt, cytochrome; DGDG, digalactosyl; DOPC, dioleoylphosphatidylcholine; ISP, Rieske [2Fe–2S] iron–sulfur protein; MGDG, monogalactosyldiacylglycerol; $MGDG_1$, monogalactosyldiacylglycerol in algal cyt b_6f that overlaps $DOPC_p$ in cyanobacterial cyt b_6f; $MGDG_2$, monogalactosyldiacylglycerol in algal cyt b_6f in the vicinity of $MGDG_1$; PDB, Protein Data Bank; PA, phosphatidic acid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; SDG, sulfoquinovosyldiacylglycerol; SQD, sulfoquinovosyldiacylglycerol; subIV, subunit IV of the b_6f complex; TMH, transmembrane helix; UDM, *n*-undecyl-β-D-maltopyranoside.

Introduction

The crystal structure of the 220-kDa dimeric cytochrome (cyt) b_6f complex, one of three hetero-oligomeric membrane protein complexes that constitute the electron transport chain of oxygenic photosynthesis, has been determined for the cyanobacteria *Mastigocladus laminosus* and *Nostoc* PCC 7120[1–4] and the green alga *Chlamydomonas reinhardtii*.[5] The arrangement of the eight polypeptide subunits and the location of the seven prosthetic groups seen in the cyanobacterial complex are shown, emphasizing the prosthetic groups (Fig. 1a) and lipids (Fig. 1b and c), in views parallel with (Fig. 1a and b; ribbon format)

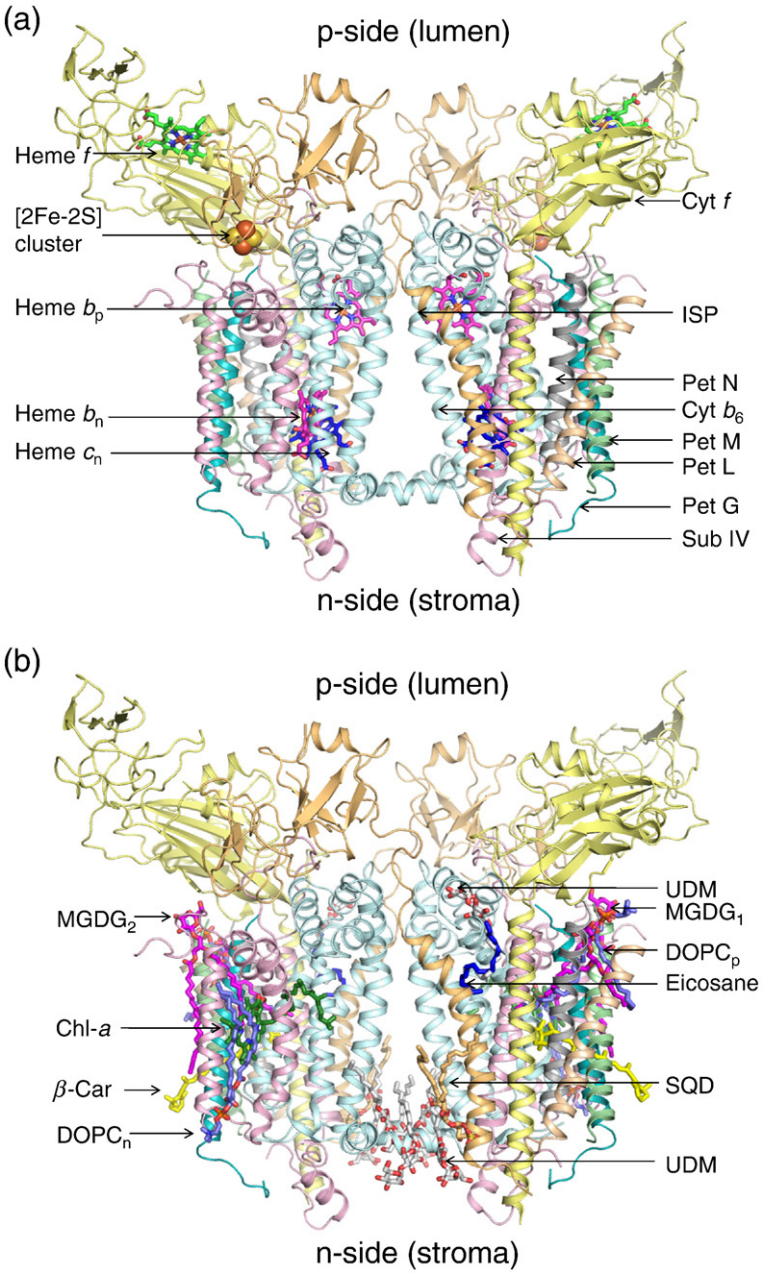

Fig. 1. Subunit organization and lipid binding sites in the cyt b_6f complex. (a) Dimeric cyt b_6f complex from *M. laminosus* (PDB ID 2E74), showing the positions of the eight protein subunits. Side view, in plane of membrane. Color code: cyt f/Pet A (yellow), cyt b_6/Pet B (cyan), Rieske Fe_2S_2 protein/Pet C (orange), subunit IV/Pet D (pink), Pet G (teal), Pet L (light brown), Pet M (green), and Pet N (gray). (b) Side view of *M. laminosus* cyt b_6f complex showing lipids, detergents, and pigments. (c) Top view along the membrane normal of *M. laminosus* cyt b_6f complex (PDB ID 2E74) showing 26 TMHs and the 2-fold symmetry axis between the monomers. Lipids ($MGDG_1$ and $MGDG_2$; magenta and red, respectively) and a pigment (eicosane; blue) from the *C. reinhardtii* b_6f complex were superimposed on the *M. laminosus* b_6f structure by combining PDB ID 2E74 and PDB ID 1Q90. The TMH of cyt b_6 (A–D) and subunit IV (E–G), ISP, cyt f, and the peripheral Pet subunits are shown as cylinders. n and p, electrochemically negative and positive sides of the complex, respectively. (d) Neutral and anionic lipids in spinach cyt b_6f complex. Major lipids of spinach b_6f complex detected by liquid chromatography with mass spectrometry.[6] (d-a) Positive ion mass spectrum of ammoniated neutral lipids $(M+NH_4)^+$ after a reverse-phase separation of a chloroform extract of cyt b_6f complex. The 764.4- and 792.6-Da species are assigned as the $(M+NH_4)^+$ ions of MGDG with 16:3, 18:3 and 18:3, and 18:3 fatty acids, respectively ($C_{43}H_{70}O_{10}$ and $C_{45}H_{74}O_{10}$; calculated mono-isotopic masses of 746.49 and 774.53 Da for the neutral species). The 926.7- and 954.6-Da species are assigned as the $(M+NH_4)^+$ ions of DGDG with 16:3, 18:3 and 18:3, and 18:3 fatty acids, respectively ($C_{49}H_{80}O_{15}$ and $C_{51}H_{84}O_{15}$; calculated mono-isotopic masses of 908.55 and 936.58 Da for the neutral species). (d-b) The negative ion mass spectrum (m/z 700–870) of the same sample is shown. The 741. 6-Da species is assigned as the $(M-H^+)^-$ ion of PG with 16:1 and 18:3 fatty acids ($C_{40}H_{71}O_{10}P_1$; calculated mono-isotopic mass of 742.48 Da for the neutral species). The ions at 815.4 and 837.6 Da are assigned as $(M-H^+)^-$ ions of SQD with either 16:0, 18:3 or 18:3, or 18:3 fatty acids, respectively ($C_{43}H_{78}O_{12}S_1$ and $C_{45}H_{76}O_{12}S_1$; calculated mono-isotopic masses of 816.51 and 838.49 Da for the neutral species).