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Conserved lipid-binding sites in membrane proteins: a focus on cytochrome c oxidase

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Abstract

Specific interactions between lipids and membrane proteins have been observed in recent high-resolution crystal structures of membrane proteins. A number of cytochrome oxidase structures were analyzed, along with many amino acid sequences of membrane-spanning regions aligned according to their location in the membrane. The results reveal conservation of lipid-binding sites and of the residues that form them. These studies imply that bound lipids have important roles that are crucial to the assembly, structure, or activity of the protein. Evidence for some of these roles in subunit interactions, membrane insertion, and protein–protein complex formation is reviewed.

Introduction

The structural and functional integrity of the biological membrane is vital to life. Besides acting as the physical barrier to define the boundaries of different cells and cellular organelles, biomembranes directly support such key cellular functions as energy production, signal transduction, and material transport. While membrane lipids provide the matrix within which membrane proteins reside and carry out these functions, the lipids also must accommodate and interact with the proteins in such a way as to maintain the integrity of the membrane and its hydrophobic barrier. A still unanswered question is how membrane proteins are specifically designed to integrate into lipid bilayers.

An impediment to answering this question has been the difficulty in obtaining high-resolution crystal structures of membrane proteins. As this bottleneck is gradually being overcome, new insights have come forth that in some cases challenge conventional wisdom and dogma. Previous efforts to address the question of lipid–protein interactions in membranes, using several powerful spectral approaches such as spin-labeling EPR and NMR, led to the conclusion that the majority of lipids, even in the protein-associated ‘annular layer’, are involved in only transitory, and relatively non-specific, interactions. Exchange rates of the lipids were observed to be fast in the time scale of most catalytic reactions, in the range of 10^7 s^{-1} , and therefore they seemed unlikely to contribute in any specific way to the functional properties of the enzymes [1]. However, the interpretation of the spectroscopic results was not unambiguous, and biochemical studies suggested that some lipids, such as cardiolipin, are strongly associated and essential for activity [2]. A well-studied model system in these types of analyses was cytochrome c oxidase [3].

Cytochrome oxidase is an essential, multisubunit, integral membrane protein found in almost all aerobic organisms on Earth. It catalyzes the final step of the electron transfer chain, the reduction of oxygen to water, while simultaneously translocating protons across the membrane

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to help generate the electrochemical gradient used for direct ATP synthesis [4]. The interplay between membrane lipids and cytochrome *c* oxidase (CcO) has been studied extensively, well before the first crystal structure of CcO became available. It has been repeatedly demonstrated that addition of lipids to detergent-purified CcO leads to 20–40% increase in activity. Moreover, the removal of cardiolipin from CcO, a lipid that is retained during purification, leads to activity loss, and re-introduction leads to its recovery [2]. Therefore, the evidence that specific lipid interactions impact function is fairly strong in the case of CcO. Lipids are found to be crucial in the purification and crystallization of other membrane proteins as well [5,6,7]. In this brief review, we present evidence that CcO does not merely present a generic hydrophobic surface to the lipid bilayer but has evolved to interact specifically with membrane lipids.

Specific interactions between lipids and CcO

With the advent of crystal structures of membrane proteins, lipids are found to occupy defined sites on the bilayer-embedded surfaces of the proteins. Not surprisingly, among these lipids is cardiolipin, which has unique structural characteristics including two phosphates and four fatty acid chains and has been implicated in the functions of a number of membrane proteins [8]. However, common lipids, such as phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC), are also resolved in a number of structures, leading to the new concept that proteins can specifically bind many different lipids in biomembranes and that such a phenomenon might, therefore, have structural or functional significance. Again, CcO crystal structures provide some of the most compelling evidence for specific lipid-binding sites that are selective for both the head groups and alkyl tails [9,10].

In a recent 1.8 Å crystal structure of the 13-subunit CcO from bovine heart mitochondria (PDB entry 2DYR), 13 complete lipid molecules are resolved, including 2 cardiolipin molecules, 1 phosphatidyl choline (PC), 3 phosphatidyl ethanolamines (PE), 4 phosphatidyl glycerols (PG), and 3 triacylglycerols (TGL) [9]. The analysis of the bovine CcO structure provides mass spectrometric data identifying not only the head groups but also the fatty acid chains, as well as crucial experiments to show that the triacylglycerol resolved in the structure is not an artifact of the purification [9]. In a slightly lower resolution structure of the 4-subunit CcO from *Rhodobacter sphaeroides* (*R.s.*) (PDB entry 1M56), 6 PE molecules are resolved [11], and in a 3.0 Å structure of the 4-subunit CcO from *Paracoccus denitrificans* (*P.d.*) (PDB entry 1QLE), 2 PC molecules are resolved [12]. The lipid molecules are found in clefts formed by transmembrane helices, often at interfaces between subunits. Two other crystal structures of the I–II subunit catalytic core of CcO (from *R.s.* at 2.0 Å resolution, PDB entry 2GSM [10], and from *P.d.* at 2.7 Å resolution, PDB entry 1AR1 [13]) show no complete lipids, but reveal well-resolved detergent molecules and partially resolved alkyl chains of lipids or detergents bound to the protein surface, in orientations and positions resembling the lipid bilayer [10].

Interestingly, an overlay of the two structures of the I–II subunit forms of CcO [10] shows that almost all the alkyl chains found in one are precisely aligned with those in the other. Further, when these bacterial oxidase structures are overlaid with the bovine CcO structure, another striking feature is observed: The positions containing alkyl chains in the bacterial oxidases correspond to the positions of fatty acid chains of phospholipid or triacylglycerol found in the mammalian enzyme. This observation indicates that these lipid and detergent molecules are not randomly arranged on the protein surface and that their binding sites are conserved.

What does a lipid-binding site look like?

The amino acid preferences for binding of lipid head groups, as observed in the crystal structures of membrane proteins, have been recently reviewed [8,14]. Detailed analyses of the

lipid head groups binding to CcO from different species seem to follow the general rules deduced from these previous studies. Molecular interactions are often found between the head groups of lipids and positively charged (K or R or H), aromatic (Y or W), or other polar residues (T or S) at the membrane interface. The hydrophobic tails of these lipids are inserted into shallow grooves on the protein surface, their presence being stabilized by precise fit through multiple van der Waals contacts, often involving hydrophobic residues such as I, V, and F. An interesting observation from the CcO crystal structures is that alkyl tails, but not their head groups, are well resolved, which has also been observed previously [15,16]. This might be a consequence of less conservation of the head group interactions (see Figure 1, Figure 3 and on-line Supplemental Figure 4), or a consequence of the crystallization conditions, which may disrupt ionic interactions between lipid head groups and polar residues in the protein. The fact that head groups of lipids are well resolved in bovine CcO could relate to the provision of extra interaction energy by the 10 additional subunits in the CcO complex.

Evolutionary conservation of lipid-binding sites in cytochrome oxidase

An examination of the structures and sequences of cytochrome oxidases from bacteria to mammals reveals that the lipid-binding sites are in fact highly conserved, even though many are formed by more than one subunit, and some are occupied by detergent molecules in the structures of bacterial CcOs. The observation that certain lipid-binding sites are conserved is not unique to CcO [8,17], but the fact that detergent-binding sites can reveal conserved lipid sites has not been previously demonstrated [10•]. One method to differentiate detergent tails from the lipid tails with unresolved head groups is to use brominated lipids. The strong X-ray scattering of bromine atoms can discriminate between them, as shown in the case of *R.s* reaction center [18•].

Cytochrome oxidase is an ancient and highly conserved enzyme [19]. The three core subunits, all encoded by the mitochondrial genome in eukaryotes, share a high degree of amino acid sequence homology among prokaryotic as well as eukaryotic species. A total of 13 different species ranging from archibacteria to human were used to analyze the degree of conservation of lipid-binding sites. In order to visualize the binding sites in sequence data, the membrane-spanning domains were aligned with the inside and outside regions properly oriented (Figure 1). The sequence alignment was based on the crystal structures and the presence of near-invariant amino acid motifs in all the oxidases. The results show that there is remarkable conservation of the residues in positions that contact lipids. Figure 1 and on-line Supplemental Figure 4 show the alignment of sequences of the two core subunits (I and II), highlighting the conserved residues that contact a lipid-binding site (within van der Waals distance). This conserved site is occupied by a PC in bovine CcO and a detergent in CcO from *R.s*.

The conservation of amino acid residues that form the grooves occupied by the alkyl tails is quite striking. By contrast, the conservation of amino acid residues associated with the head group association is less so (see also on-line Supplemental Figure 4), consistent with less resolution in X-ray structures. This would suggest that the binding and positioning of alkyl chains is more important for the assembly, structure, or function of CcO than the exact positioning of the headgroup.

In one case, however, defined lipid sites do not appear to be strongly conserved. The thermophilic CcO from *Thermus thermophilus* is structurally and functionally highly similar to the other *aa₃*-type oxidases, but the degree of conservation of residues in lipid sites is significantly less, compared with that of the other species (Figure 1 and on-line Supplemental Figure 4), and a high-resolution structure at 2.3 Å (PDB entry 1XME [20] reveals no associated alkyl chains. This observation might be rationalized by the fact that lipids at high temperatures may be too mobile to play a structural role in the enzyme. Thus, the thermophilic enzyme may

be designed to maintain its structure and accomplish its function without defined partnerships with lipids.

Functional significance

Such high degree of conservation suggests important structural, functional, or assembly roles of the bound lipid, but studies that precisely define these roles are few. One role for which there is some evidence is the stabilization of interactions between subunits in multisubunit complexes. Among the conserved lipid-binding sites in CcO, there are two that have been tested by mutational analysis and show characteristics that support such a role. The first site is at the bottom of the V-shaped cleft formed by two helix bundles of subunit III, close to the interface with subunit I (Figure 2a), and the other site is at the interface of subunits I, II, and III (Figure 2b).

The lipid shown in Figure 2a has been studied by mutagenesis of conserved residues that interact with either the headgroup (R226_{III}A) or the alkyl tail (W59_{III}A-F86_{III}A). These mutant forms show normal or below-normal activities but in both cases display an increased tendency to undergo suicide inactivation [21••], commonly observed for subunit-III-less CcO [22] even though subunit III is retained. This lipid molecule is normally involved in a tight network of bonds that connect it, through a second lipid, to subunit I. It is postulated that this subunit–lipid–subunit interaction is important in maintaining a conformation of subunit I that prevents side reactions from occurring at the oxygen-reduction site [21••].

At the other site (Figure 2b), the phosphodiester of the lipid head group is involved in interactions with subunits I, II, and III. Mutation of any of the highly conserved residues that are involved in that interaction, aspartate 271_I, arginine 234_{II}, or lysine 103_{III}, leads to mutant forms of CcO that tend to dissociate during purification (Hosler, Florens, Qian, unpublished results). This suggests a role for this lipid in maintaining the structural integrity of the complex by mediating a key subunit–subunit–subunit interaction.

Another role for lipid that has experimental support is mediation of interactions between protein complexes. The unusual lipid, cardiolipin, has been resolved in the bovine CcO structure at the homodimer interface [9••]. In addition, there is evidence that cardiolipin facilitates heterodimer formation between cytochrome *bc*₁ and cytochrome *c* oxidase. Studies in yeast where amounts of cardiolipin are varied indicate a correlation between the level of this lipid and the level of the *bc*₁-*aa*₃ supercomplex [23,24].

Specifically bound lipids may also act as a sealant that allows proteins to integrate into the bilayer without disrupting the integrity of the membrane. In this regard, an interesting observation is that one of the two fatty acid chains in many of the conserved lipid sites appears to be more tightly bound and more highly conserved than the other. This suggests a possible anchoring strategy: A seamless interface could be produced by sharing the lipid between the protein and the bilayer. The PC-binding site shown in Figure 3 illustrates this property. The oleoyl chain is less integrated into the protein and so capable of participating in a shared interface with the lipid bilayer.

The hypothesis that membrane lipids can function as molecular chaperones during the folding and assembly process of membrane proteins is relatively new. However, there is compelling evidence that PE is required in the correct folding and insertion of lactose permease (LacY) in *E. coli* [25,26] and that the function and correct topology of LacY in PE-deficient strain of *E. coli* can be restored by a foreign lipid species monoglucosyl diacylglycerol, which has similar physical and chemical properties [27•]. No requirements for particular lipid species for CcO folding and maturation have yet been demonstrated. In fact, in the authors' laboratory, expression of native, active CcO has been found to be unaffected in mutant strains of *R.*

sphaeroides lacking several different lipids (C Hiser, B Tamot, X Zhang, C Benning, S Ferguson-Miller, unpublished results).

Conclusions

In general, evidence is accumulating that specific lipids play important roles in membrane proteins, but the big picture is not yet clear as to how, and how specifically, lipids interact with and enable membrane proteins to achieve their full functionality. Further high-resolution crystal structures, as well as biochemical and mutational analyses, are needed to understand lipid roles and to utilize the knowledge in protein design and engineering.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Marsh D, Horvath LI. Structure, dynamics and composition of the lipid-protein interface. Perspectives from spin-labeling. *Biochimica et Biophysica Acta, Rev Biomembr* 1998;1376:267–296.
 2. Robinson NC, Zborowski J, Talbert LH. Cardiolipin-depleted bovine heart cytochrome *c* oxidase: binding stoichiometry and affinity for cardiolipin derivatives. *Biochemistry* 1990;29:8962–8969. [PubMed: 2176838]
 3. Powell GL, Knowles PF, Marsh D. Spin-label studies on the specificity of interaction of cardiolipin with beef heart cytochrome oxidase. *Biochemistry* 1987;26:8138–8145. [PubMed: 2831938]
 4. Hosler JP, Ferguson-Miller S, Mills DA. Energy transduction: proton transfer through the respiratory complexes. *Annu Rev Biochem* 2006;75:165–187. [PubMed: 16756489]
 5. Zhang H, Kurisu G, Smith JL, Cramer WA. A defined protein–detergent–lipid complex for crystallization of integral membrane proteins: the cytochrome b6f complex of oxygenic photosynthesis. *Proc Natl Acad Sci USA* 2003;100:5160–5163. [PubMed: 12702760]
 - 6•. Guan L, Smirnova IN, Verner G, Nagamori S, Kaback HR. Manipulating phospholipids for crystallization of a membrane transport protein. *Proc Natl Acad Sci USA* 2006;103:1723–1726. [PubMed: 16446422] By manipulating phospholipid contents in various lactose permease preparations, the authors are able to obtain different crystal forms with different X-ray diffraction quality.
 7. Warne T, Schertler GFX. The purification of G-protein coupled receptors for crystallization. *Struct Biol Membr Protein* 2006;51–71.
 8. Palsdottir H, Hunte C. Lipids in membrane protein structures. *Biochimica et Biophysica Acta* 2004;1666:2–18. [PubMed: 15519305]
 - 9••. Shinzawa-Itoh K, Aoyama H, Muramoto K, Terada H, Kurauchi T, Tadehara Y, Yamasaki A, Sugimura T, Kurono S, Tsujimoto K, et al. Structures and physiological roles of 13 integral lipids of bovine heart cytochrome *c* oxidase. *EMBO J* 2007;26:1713–1725. [PubMed: 17332748] This paper describes the use of mass spectrometry to understand the detailed structures of the 13 lipids

resolved in the bovine CcO structure, including the exact chain lengths and positions of the unsaturated bonds.

- 10••. Qin L, Hiser C, Mulichak A, Garavito RM, Ferguson-Miller S. Identification of conserved lipid/detergent-binding sites in a high-resolution structure of the membrane protein cytochrome *c* oxidase. *Proc Natl Acad Sci USA* 2006;103:16117–16122. [PubMed: 17050688] This paper presents a 2.0 Å structure of the catalytic core of *RsCcO*, as well as the finding that certain detergents with the right headgroup and tail characteristics can mimic lipids and occupy lipid-binding sites on membrane proteins.
11. Svensson-Ek M, Abramson J, Larsson G, Tornroth S, Brzezinski P, Iwata S. The X-ray crystal structures of wild-type and EQ(I-286) mutant cytochrome *c* oxidases from *Rhodobacter sphaeroides*. *J Mol Biol* 2002;321:329–339. [PubMed: 12144789]
12. Harrenga A, Michel H. The cytochrome *c* oxidase from *Paracoccus denitrificans* does not change the metal center ligation upon reduction. *J Biol Chem* 1999;274:33296–33299. [PubMed: 10559205]
13. Ostermeier C, Harrenga A, Ermler U, Michel H. Structure at 2.7 Å resolution of the *Paracoccus denitrificans* two-subunit cytochrome *c* oxidase complexed with an antibody Fv fragment. *Proc Natl Acad Sci USA* 1997;94:10547–10553. [PubMed: 9380672]
14. Hunte C. Specific protein–lipid interactions in membrane proteins. *Biochem Soc Trans* 2005;33:938–942. [PubMed: 16246015]
15. Luecke H, Schobert B, Richter HT, Cartailler JP, Lanyi JK. Structure of bacteriorhodopsin at 1.55 Å resolution. *J Mol Biol* 1999;291:899–911. [PubMed: 10452895]
16. Murata T, Yamato I, Kakinuma Y, Leslie AGW, Walker JE. Structure of the rotor of the V-Type Na⁺-ATPase from *Enterococcus hirae*. *Science (Washington, DC, USA)* 2005;308:654–659.
17. Wakeham MC, Sessions RB, Jones MR, Fyfe PK. Is there a conserved interaction between cardiolipin and the type II bacterial reaction center? *Biophys J* 2001;80:1395–1405. [PubMed: 11222300]
- 18•. Roszak AW, Gardiner AT, Isaacs NW, Cogdell RJ. Brominated lipids identify lipid binding sites on the surface of the reaction center from *Rhodobacter sphaeroides*. *Biochemistry* 2007;46:2909–2916. [PubMed: 17315985] Externally added brominated phospholipids are used to distinguish between tails of lipids and tails of detergents in the case of the reaction center from *R. sphaeroides*
19. Castresana J, Luebben M, Saraste M, Higgins DG. Evolution of cytochrome oxidase, an enzyme older than atmospheric oxygen. *EMBO J* 1994;13:2516–2525. [PubMed: 8013452]
20. Hunsicker-Wang LM, Pacoma RL, Chen Y, Fee JA, Stout CD. A novel cryoprotection scheme for enhancing the diffraction of crystals of recombinant cytochrome *ba3* oxidase from *Thermus thermophilus*. *Acta Crystallogr, Section D: Biol Crystallogr* 2005;D61:340–343. [PubMed: 15735345]
- 21••. Varanasi L, Mills D, Murphree A, Gray J, Purser C, Baker R, Hosler J. Altering conserved lipid binding sites in cytochrome *c* oxidase of *Rhodobacter sphaeroides* perturbs the interaction between subunits I and III and promotes suicide inactivation of the enzyme. *Biochemistry* 2006;45:14896–14907. [PubMed: 17154527] The first mutational studies of the conserved lipid-binding sites in CcO show that the disruption of lipid interactions lead to an altered binding of subunit III and enzyme complex that displays suicide inactivation.
22. Bratton MR, Pressler MA, Hosler JP. Suicide inactivation of cytochrome *c* oxidase: catalytic turnover in the absence of subunit III alters the active site. *Biochemistry* 1999;38:16236–16245. [PubMed: 10587446]
23. Pfeiffer K, Gohil V, Stuart RA, Hunte C, Brandt U, Greenberg ML, Schaeffer H. Cardiolipin stabilizes respiratory chain supercomplexes. *J Biol Chem* 2003;278:52873–52880. [PubMed: 14561769]
24. Zhang M, Mileyskova E, Dowhan W. Cardiolipin Is essential for organization of complexes III and IV into a supercomplex in intact yeast mitochondria. *J Biol Chem* 2005;280:29403–29408. [PubMed: 15972817]
25. Bogdanov M, Dowhan W. Lipid-assisted protein folding. *J Biol Chem* 1999;274:36827–36830. [PubMed: 10601231]
26. Bogdanov M, Heacock PN, Dowhan W. A polytopic membrane protein displays a reversible topology dependent on membrane lipid composition. *EMBO J* 2002;21:2107–2116. [PubMed: 11980707]

- 27•. Xie J, Bogdanov M, Heacock P, Dowhan W. Phosphatidylethanolamine and monoglucosyldiacylglycerol are interchangeable in supporting topogenesis and function of the polytopic membrane protein lactose permease. *J Biol Chem* 2006;281:19172–19178. [PubMed: 16698795] The paper shows that LacY function and correct topology can be restored in a PE-deficient strain of *E. coli*, by a foreign lipid, monoglucosyldiacylglycerol, which is similar to PE in terms of its non-bilayer prone property, lack of net charge, and the ability of its headgroup to form hydrogen bonds.

KEY:
Species	a.a. No.	Inner loop	Transmembrane region	Outer loop	a.a. No.
<i>E. Coli</i>	304	→ TFSRKR	LF [□] GYTSLVWATVCITVLSFIVWLHHFTMG	A	340
	376	← VIRGQ	YMTFLWNFIKVGTPIAIIMTTIGFFANVNA	G	341
<i>T. Thermus</i>	254	→ KQAGGK	LVSDPMARLAFLLFLLSTFVGFHHQFADP	GI	290
	327	← RLRGAF	ELSAAVTFATMLSPVAVFLTLVSHIMKWTP	D	291
Cyanobacter	286	→ KP	LF [□] GYRVVAISTVAIGVIGFTVWAHHMFTSG	T	318
	352	← KGG	WITAVWNFVKIGTPVAITMSTIMFFLRLWD	P	
<i>Rhodobacter</i>	308	→ KP	IF [□] GYLPMVYAMVAIGVLGFVVAHHMYTAG	L	340
	374	← SGG	WMTAIWS [□] FIKIGTPVAIVMTAMMFYSQOTL	S	341
<i>Rickettsia</i>	284	→ KP	IF [□] GYQGMVGAMVIIGFVGFVWAHHMFTVG	L	316
	350	← SGG	WMTAIWS [□] FIKIGTPVAIIMTGATFYILANY	S	317
Coral	266	→ KQH	IF [□] GYLGMVYAMISIGVLGFVVAHHMFTVG	M	299
	333	← SGG	YITAIWS [□] FIKIGTPVAIIMTAATFYARTDV	D	300
<i>Choanoflagulla</i>	267	→ KP	VFGYLGVMYAMLSIGLLGFVVAHHMYTVG	M	299
	333	← SGG	FMTAIWS [□] FIKIGTPVAIIMTAATFYARTDV	D	300
Coelacanth	264	→ KKE	PEGYMGVMWAMMAIGLLGFVVAHHMFTVG	M	297
	331	← VGG	HLTAIWS [□] VKVGTPIAIIMTASTFYARTDV	D	298
Frog	264	→ KKE	PEGYMGVMWAMMSIGLLGFVVAHHMFTVD	L	297
	331	← TGG	HMTAIWS [□] VKVGTPIAIIMTASTFYARTDV	N	298
Platypus	264	→ KKE	PEGYMGVMWAMMSIGFLGFVVAHHMFTVG	M	297
	331	← DGG	HLTAIWS [□] VKVGTPIAIIMTASTFYARTDV	D	298
Bovine	264	→ KKE	PEGYMGVMWAMMSIGFLGFVVAHHMFTVG	M	297
	331	← NGG	HLTAIWS [□] VKVGTPIAIIMTASTFYARTDV	D	298
Slow loris	264	→ KKE	PEGYMGVMWAMMSIGFLGFVVAHHMFTVG	M	297
	331	← NGG	HLTAIWS [□] VKVGTPIAIIMTASTFYARTDV	D	298
Human	264	→ KKE	PEGYMGVMWAMMSIGFLGFVVAHHMFTVG	M	297
	331	← NSG	HLTAIWS [□] VKVGTPIAIIMTASTFYARTDV	D	298

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Figure 1.

Conservation of amino acids of subunit I at the phosphatidyl choline (PC)/decyl maltoside (DM) binding site. Conserved residues that form a lipid-binding site in evolutionarily distant organisms are shown in the transmembrane helices VII and VIII of subunit I. Amino acids have been color coded to show which bind to PC (red) or to the detergent decyl maltoside (cyan) (see key at the top). Amino acids that are conserved and bind directly to the lipid/detergent alkyl chains are indicated in solid colors and semi-conserved residues are boxed. It is clear that other residues in the vicinity are also highly conserved (e.g. bov W323, *R.s.* W366). The bovine crystal structure (PDB entry 2DYR) reveals that five conserved residues of subunit I bind the palmitoyl chain (F268, S322, L324, A325, H328) and one conserved residue binds both the palmitoyl and oleoyl chains (F321). Many of these same residues are involved in binding of the alkyl chain of DM in *R.s.* oxidase. The conserved residues in subunit II, which share the same PC site, are shown in on-line Supplemental Figure 4. The exact positioning of these residues in the vicinity of PC and DM are shown in Figure 3.

Figure 2.

Conserved lipid-binding sites seen in the structural overlay of *R.s.CcO* (PDB entry 1M56) bovine *CcO* (PDB entry 2DYR) and *P.d.CcO* (PDB entry 1QLE). The protein-surface representation is of the *R.s.CcO* structure and is colored by different subunits (subunit I: wheat; subunit II: magenta; subunit III: gray). (a). In the V-shaped cleft of subunit III, a PE (green), a PG (yellow), and a PC (cyan) are resolved in *R.s.CcO*, bovine *CcO*, and *P.d.CcO*, respectively. Not shown is a second lipid molecule resolved in both the *R.s.* and bovine *CcO*, which mediates interaction between the lipid shown and subunit I behind. Conserved residues that interact with the headgroup (R226_{III}), and the tails (W59_{III} and F86_{III}) of the lipid are shown in sticks, colored by atom type, and labeled by *R.s.CcO* numbering. (b). At the interface of subunit I, II, III, a PE (green), a PG (yellow), and a PC (cyan) are resolved in the structures of *R.s.CcO*, bovine *CcO* and *P.d.CcO*, respectively. Conserved residues that interact with the lipid headgroup directly or via crystallographically resolved water (red sphere) are shown in sticks and labeled by *R.s.CcO* numbering.

Figure 3.

Overlay of the amino acid residues in bovine and *R.s.CcO* that ligate phosphatidyl choline and decyl maltoside. The color coding used is the same as in Figure 1 and on-line Supplemental Figure 4. **(a and c)** show superimposed conserved amino acid residues that form the PC/DM-binding site, from bovine and the *R.s.CcO* crystal structures. **(b)** Shows how the DM alkyl chain in *R.s.CcO* occupies the same position as the inner, palmitoyl chain of the bovine PC molecule. The lower three panels **(d-f)** show the end-on views of **(a-c)**. When examined with the sequence data shown in Figure 1 (and on-line Supplemental Figure 4), it is clear that the conservation of amino acids in the region of the alkyl tails is much more pronounced than in the region of the headgroup and that the buried palmitoyl chain of PC (position 1), also occupied by the DM decyl chain, is more strongly bound by conserved residues than the externally located oleoyl chain.