E. coli cell-free system supplemented with *E. coli* cell membrane fractions, heme A and Cu_2SO_4 (about 500 μg of the proteins/ml was produced). Blue native PAGE and SDS-PAGE of dodecylmaltoside-solubilized ^{35}S-labeled proteins revealed a protein complex with a normal subunit stoichiometry. Synthesized proteins were purified by a MonoQ column chromatography, giving a fraction exhibiting the KCN sensitive ferrocytochrome *c* oxidation activity and the normal Soret/visible spectra. The elution volume of the fraction identical to that of the authentic enzyme also suggests the native conformation of the synthesized enzyme. These results indicate successful cell-free synthesis of the native enzyme. However, the amount of the functional enzyme was still about 1% of the synthesized proteins.

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S11.24 Mutations of possible proton-transfer pathways of bovine heart cytochrome *c* oxidase

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X-ray structures of the bovine enzyme at 1.8–1.9 Å resolution show a possible proton-pumping pathway (H-pathway) composed of a hydrogen bond network and a water channel. The former includes the peptide bond, which could facilitate unidirectional proton transfer. Mutant enzymes in which proline is introduced to block proton transfer through the peptide bond and in which bulkier amino acids are introduced to the water channel to block its water-transfer function showed complete abolishment of the proton pumping without affecting the dioxygen reduction activity. These results and the previous proton pumping site mutation (Asp51Asn) result strongly support the proposed proton pumping function of H-pathway. On the other hand, a mutation (Asn98Asp in bovine numbering) of bacterial enzymes in D-pathway, a possible proton-transfer pathway connecting the negative-side surface with the dioxygen reduction site, abolishes proton pumping without impairing the O_2 reduction activity, suggesting that D-pathway of bacterial enzymes convey pumping-protons also. However, the Asn98Asp mutant of the bovine D-pathway negligibly influenced the O_2 reduction and proton pumping (H^+/e^-, 0.64 (n=8) for wild type and 0.65 (n=7) for the mutant). The present results suggest that function of D-pathway is not conserved between bovine and bacterial enzymes.

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S11.25 X-ray structure of carbon monoxide at copper site of the dinuclear site of cytochrome *c* oxidase

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The copper site (Cu_B) in the O_2 reduction site of cytochrome *c* oxidase is silent to most of spectroscopic techniques and thus the role of Cu_B in the O_2 reduction mechanism is poorly understood. The fully reduced carbon monoxide (CO) derivative of bovine heart cytochrome *c* oxidase photolyzed below 140 K shows C–O stretch band at 2062 cm^{-1}, assignable to CO bound at Cu_B. However, the infrared result provides no direct geometric information for the bound CO. Electron density map of the fully reduced-CO bound form of bovine heart cytochrome *c* oxidase under light conditions at 100 K at 1.8 Å resolution shows an electron density peak assignable to CO near Cu_B atom. The F_o–F_c map strongly suggests a side-on binding of CO to Cu_B, although the possibility of an end-on binding cannot be excluded at this resolution. The distances between Cu_B and the two atoms of CO are 2.5 Å and 2.4 Å, suggesting a fairly weak metal/ligand interaction. The weak interaction is likely to contribute the stability of the oxygenated form of the enzyme (Fe_{a3}–O_2), which is prerequisite for the four electron reduction of O_2 at Fe_{a3}.

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S11.26 Structural analyses for lipid/protein interactions in bovine heart cytochrome *c* oxidase

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All 13 lipids, including two cardiolipins, one phosphatidylcholine, three phosphatidylethanolamines, four phosphatidylglycerols and three triglycerides were identified in a crystalline bovine heart cytochrome *c* oxidase (C*c*O) preparation. The chain lengths and unsaturated bond positions of the fatty acid moieties determined by mass spectrometry suggest that each lipid head group identifies its specific binding site within C*c*O. Binding of dicyclohexylcarbodiimide to the O_2-transfer pathway of C*c*O causes two palmitate tails of phosphatidylglycerols to block the pathway, suggesting that the palmitates control the O_2 transfer. The phosphatidylglycerol with vaccenate (*cis*-Δ^{11}-octadecenoate) was found in C*c*O of *Paracoccus denitrificans*, a possible ancestor of mitochondrion. This indicates that the vaccenate is conserved in bovine C*c*O in spite of the abundance of oleate (*cis*-Δ^9-octadecenoate). The X-ray structure indicates that the protein moiety selects *cis*-vaccenate against *trans*-vaccenate for the O_2-transfer pathway. These results suggest that vaccenate plays a critical role in the O_2-transfer mechanism and that the lipid binding specificity is determined by both the head group and the fatty acid tail.

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S11.27 X-ray structural analysis of zinc/cadmium inhibitory site in bovine heart cytochrome *c* oxidase

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