PROTEIN-LIPID INTERACTIONS WITHIN PURIFIED AND RECONSTITUTED CYTOCHROME c REDUCTASE AND OXIDASE

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Analysis of the rotational mobility of spin-labeled bovine heart cytochrome c oxidase by saturation transfer electron paramagnetic resonance (ST-EPR) has recently demonstrated that this enzyme complex can be obtained as an aggregate of individual protein molecules which display little or no rotational mobility on the submillisecond time scale (1). Aggregation appears to occur as a result of protein-protein interactions, as rotationally mobile \( \tau_\alpha = 1 \times 10^{-7} \) s enzymes can be isolated by further purification of the complex to remove contaminating polypeptides, followed by gel filtration to separate aggregates from individual enzyme molecules. This tendency of cytochrome oxidase to exist as protein “patches” may reflect the situation within the mitochondrial inner membrane (2).

Correlation of information gained on the rotational mobility of cytochrome oxidase to the fluidity of lipid adjacent to the protein indicates that an immobilized or “boundary” lipid population is only detectable, using EPR, when the protein moiety of the complex is immobilized on the submillisecond time scale (1). This presumably is due to the formation of enzyme aggregates resulting in the entrapment of spin-labeled lipid between cytochrome oxidase molecules. Steady-state activity displayed by cytochrome oxidase is unaffected by the rotational mobility, and therefore the aggregation state, of either the purified or reconstituted complex. Furthermore, electron transport rates are not influenced by the fluidity of the hydrophobic environment adjacent to the protein.

In the present study, the rotational mobility of another mitochondrial electron transport complex, ubiquinol-cytochrome c oxidoreductase (1.10.2.2), has been studied by ST-EPR and correlated with lipid fluidity adjacent to the purified and reconstituted protein. Because both cytochrome c reductase and oxidase interact with cytochrome c, the only water-soluble protein component of the electron transport chain, it was of interest to investigate whether cytochrome c reductase also possessed the type of aggregation behavior displayed by oxidase, and whether the previous conclusion, that immobilized lipid populations are EPR-detectable only when the protein is immobilized, could be further substantiated.

MATERIALS AND METHODS

All experimental procedures utilized in this study have been previously specified (1) with the exception that bovine heart mitochondrial ubiquinol-cytochrome c oxidoreductase was isolated according to Rieske (3), and enzymic activity assayed by the method of Speck et al. (4). Protein rotational mobility was studied employing 4-maleimido-2,2,6,6-tetramethylperidinoxyxyl (MSL), while boundary lipid fluidity was assayed with 2-(14-carboxytetradecyl-N-ethylmaleic ester)-2-ethyl-4,4-dimethyl-3-oxazolidinylxoxyl (ML 1, 14), which was kindly provided by P. Devaux.

RESULTS AND DISCUSSION

Labeling of cytochrome c reductase with 2 mol MSL/mol enzyme results in a first harmonic spectrum \( V_h \) possessing a minimal population of weakly immobilized spins detectable at 4°C after the enzyme has been chromatographed through Sephadex G-25 to eliminate noncovalently bound spin label. Approximately 1 mol of MSL covalently binds/mol reductase during the 15-min incubation period at 20°C. Therefore treatment of this enzymic complex with 1 mM cysteine and 10 mM ferricyanide, which was necessary to diminish the weakly immobilized spin population in the case of spin-labeled cytochrome oxidase, was unnecessary. Furthermore, spin-labeled cytochrome c reductase chromatographs as a single species and does not aggregate on the gel filtration column as commonly occurs with oxidase. ST-EPR spectra \( V_h \) of the purified enzyme in a detergent-containing buffer (50 mM KH2PO4, 0.25% Tween 20, pH 7.5) are representative of a highly mobile protein \( \tau_\alpha = 4 \times 10^{-7} \) s. Thus, a key difference between the reductase and oxidase enzymes appears to be the degree to which they self-associate. Table I shows that incorporation of spin-labeled cytochrome c reductase into a membrane bilayer, using the reconstitution techniques of either cholate dialysis or soni-
cation (1) at a lipid:protein ratio of 12.5:1 (by weight), results in a $V'_2$ spectrum characteristic of a rotationally mobile membrane protein ($T_2 = 7 \times 10^{-5}$s).

Because cytochrome reductase was obtained only as a rotationally mobile, and presumably disaggregated, protein, it was of interest to investigate whether lipid adjacent to the protein was mobile or not. To perform this experiment, we employed the MSL(1, 14) spin probe in which the nitrooxide group is attached at one end of the acyl chain and the other end reacts with sulphydryl groups through an N-ethylmaleimide linkage (5). MSL(1, 14) was added at a molar ratio of 2:1 to cytochrome reductase and noncovalently bound probe eliminated as previously described (1). As Table I also indicates, the hydrophobic environment adjacent to the protein was fluid, although an immobile component was also usually observed.

Our results demonstrate that mobile boundary lipid populations are detected by EPR when the protein moiety of either the reductase or oxidase complexes is rotationally mobile. Of course, a difficulty with these experiments arises from the fact that we do not know whether the protein environment is being uniformly sampled by the MSL(1, 14) probe.

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REFERENCES


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**FATTY ACID PERTURBATION OF A MEMBRANE PROTEIN-LIPID INTERACTION**

**A Terbium (Tb$^{3+}$) Fluorescence Study**

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Recent studies suggest that immiscible lipid domains are an important structural feature of plasma membranes (1). By preferentially partitioning into a particular domain, free fatty acids (FFA) were found to alter lipid structure differentially, depending upon whether they are cis or saturated. These results, together with observed differential effects of FFA on membrane function (2, 3) suggest that FFA may, by means of protein-lipid interactions, alter the structure of proteins in particular domains. To test this hypothesis we chose to use Tb$^{3+}$ fluorescence as a means of monitoring protein structural alteration as a function of FFA exposure. The rationale for using Tb$^{3+}$ relies on the following: (a) significant Tb$^{3+}$ fluorescence occurs only by energy transfer from a donor fluorophore, which from the observed excitation spectrum was shown in our case to be tryptophan; (b) Tb$^{3+}$ has been shown to substitute for Ca$^{2+}$ in many calcium binding proteins; and (c) it has been suggested that a calcium binding protein in lymphocyte plasma membranes is perturbed by exposure to cis but not saturated FFA (2).