ESCHERICHIA COLI PYRUVATE OXIDASE

INTERACTION OF A PERIPHERAL MEMBRANE PROTEIN WITH LIPIDS

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Pyruvate oxidase is one of several membrane-associated flavoprotein dehydrogenases that feed electrons into the *Escherichia coli* aerobic respiratory chain. Unlike the other flavoprotein dehydrogenases from *E. coli* , it is a peripheral membrane protein and can be released from the membrane by sonication. The purified enzyme is a tetramer of identical subunits. Each subunit contains a tightly bound flavin adenine dinucleotide (FAD) coenzyme. The addition of a second cofactor, thiamin pyrophosphate (TPP), is required to elicit catalytic activity. The reaction catalyzed is the oxidative decarboxylation of pyruvate to acetate and carbon dioxide. The purified enzyme is free of lipids.

The enzymatic activity of pyruvate oxidase is stimulated ~ 25-fold by the addition of any of a wide variety of amphiphiles, including phospholipids, to the assay mixture. The affinity between a charged detergent and pyruvate oxidase increases with the length of its hydrocarbon chain, suggesting that the mode of interaction between the protein and the amphiphile is primarily hydrophobic (1). In addition to the dramatic effect on the turnover number, the presence of lipid alters the K_m and Hill coefficients for substrate and cofactor (2). Equilibrium binding studies with TPP have shown that the influence of lipids and detergents on the K_m and Hill coefficients of this cofactor directly reflects changes in its equilibrium binding isotherm (3). Thus, the activity of this enzyme can be modulated by lipids through alteration of the strength and cooperativity of substrate and cofactor binding as well as by changing the turnover number.

Not only do lipids have a dramatic influence on the catalytic events involving substrate and cofactor, but the catalytic ligands also have a strong influence on the affinity of the protein for lipids, as required by thermodynamic reciprocity. Several experiments have indicated an energetic and conformational coupling between the catalytic active site and the lipid binding site on pyruvate oxidase (2, 4, 5). The bound FAD is reduced in the presence of pyruvate and TPP. This reduction of the flavoprotein is accompanied by a conformational change that alters the solution behavior of the protein and exposes a region of the protein to proteolytic attack. The reduced form of the enzyme has a tendency to self-aggregate, is highly surface labile, and manifests a higher affinity for amphiphiles. Yet no major alterations in the secondary structure of the protein, as determined by circular dichroism, accompany flavin reduction. A graphic demonstration of the influence of substrate and cofactor upon lipid affinity is provided by ultracentrifugation experiments with pyruvate oxidase and dipalmitoylphosphatidylcholine vesicles. In the absence of the catalytic ligands, the protein and lipid vesicles sediment separately through a sucrose gradient; in their presence, most of the protein and phospholipid migrate as a single species (5). Conceivably, pyruvate oxidase is bound to the bacterial inner membrane in vivo only when the concentrations of substrate and cofactor are sufficiently high.

Rapid kinetics techniques have been used to study the effect of lipid activators on isolated portions of the enzymatic cycle. The rate of decarboxylation was determined by monitoring the release of CO_2 in a rapid mixing chemical quench experiment. The rates of reduction and reoxidation of the FAD moiety were followed in stopped-flow experiments. Heavy atom isotope experiments established that the actual decarboxylation is not rate limiting, either in the presence or the absence of lipid activator. By comparing the rates of these portions of the overall cycle, we determined that it is an early step in the catalytic cycle, prior to decarboxylation, which is accelerated by amphiphilic activators. This step may be the addition of TPP to pyruvate at the active site.

Experiments utilizing controlled proteolysis of pyruvate oxidase have provided additional insight into the interaction of the enzyme with lipids. Reduction of the protein by pyruvate and TPP allows the cleavage of the enzyme by endoproteases at a site 4,000 daltons from the COOH-terminus. Such cleavage results in loss of the amphiphile-binding properties characteristic of the native enzyme. Addition of amphiphile prior to protease protects the enzyme against cleavage (6). Furthermore, cleavage of reduced enzyme activates the enzyme ~ 25-fold, just as does incubation with lipids. This evidence suggests that there is a common hydrophobic region of the peptide that contains the sites of proteolytic cleavage and lipid binding.

Demonstration of the physiological significance of the interaction of lipids with pyruvate oxidase comes from experiments with bacterial membranes. When pyruvate oxidase is mixed with *E. coli* inner membranes, which are devoid of pyruvate oxidase but contain a functional electron transport chain, pyruvate-supported oxygen consumption is reconstituted (7). In the absence of oxygen, all cytochromes in this reconstituted system are rapidly reduced by pyruvate via the enzyme. However, reconstitution and cytochrome reduction cannot be achieved with proteolytically activated pyruvate oxidase, even though this form of the enzyme rapidly reduces soluble electron acceptors such as ferricyanide. Apparently the same structural features of the enzyme that promote its interaction with lipids are important in its interaction with the membrane-bound electron transport chain.

The study of the protein-lipid interactions of pyruvate oxidase has been particularly fruitful. Consideration of the reciprocal effects between the catalytic ligands and lipid activators leads to the key point that protein-lipid interactions can be allosterically controlled in a manner similar to that already well documented for interactions between proteins and metabolites and for protein-protein interactions.

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REFERENCES

1. Blake, R., L. P. Hager, and R. B. Gennis. 1978. Activation of pyruvate oxidase by monomeric and micellar amphiphiles. *J. Biol. Chem.* 253: 1963–1971.
2. Cunningham, C. C., and L. P. Hager. 1971. Crystalline pyruvate oxidase from *Escherichia coli* III. Activation by phospholipids. *J. Biol. Chem.* 246:1583–1589.
3. O'Brien, T. A., R. Blake, and R. B. Gennis. 1977. Regulation by lipids of cofactor binding to a peripheral membrane enzyme: binding of thiamin pyrophosphate to pyruvate oxidase. *Biochemistry.* 16:3105–3109.
4. Russell, P., H. L. Schrock, and R. B. Gennis. 1977. Lipid activation and protease activation of pyruvate oxidase: evidence suggesting a common site of interaction on the protein. *J. Biol. Chem.* 252:7883–7887.
5. Schrock, H. L., and R. B. Gennis. 1980. Specific ligand enhancement of the affinity of *E. coli* pyruvate oxidase for dipalmitoyl phosphatidylcholine . *Biochim. Biophys. Acta.* 614:215–220.
6. Russell, P., L. P. Hager, and R. B. Gennis. 1977. Characterization of the proteolytic activation of pyruvate oxidase: control by specific ligands and by the flavin oxidation-reduction state. *J. Biol. Chem.* 252:7877–7882.
7. Williams, F. R., and L. P. Hager. 1960. Solubilization and enzymatic activity of bacterial cytochrome *b*. *Biochim. Biophys. Acta.* 38:566–567.

STUDIES ON THE ENERGY-TRANSDUCING ATPase COMPLEX OF BIOLOGICAL MEMBRANES

Cross Hybrid Reconstitution of F_1 and F_0 of *Escherichia coli* Plasma Membrane and Rat Liver Mitochondria

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Recently, we were able to demonstrate hybrid reconstitution between F_1 from rat liver mitochondria and the F_0-membrane section of human liver carcinoma mitochondria (1). Some earlier papers indicated that F_1 from Baker's yeast mitochondria could bind to F_1-depleted SMP from beef heart and partially restore the ATPase activity and other energy coupling reactions (2). Soluble BF_1 from different mutants of *Streptococcus faecalis* and *Escherichia coli* were also found to be interchangeable (3). Here, we present further evidence of the successful cross-hybrid reconstitution of F_1 and F_0 from *E. coli* plasma membrane and rat liver mitochondria. The energy-transducing reactions of *E. coli* plasma membrane and their regulation by changes in membrane fluidity are also reported.

METHODS

The wild-type B strain of *E. coli* was grown at 37°C in a minimal salt medium. Soluble BF_1 and plasma membranes were prepared by a

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