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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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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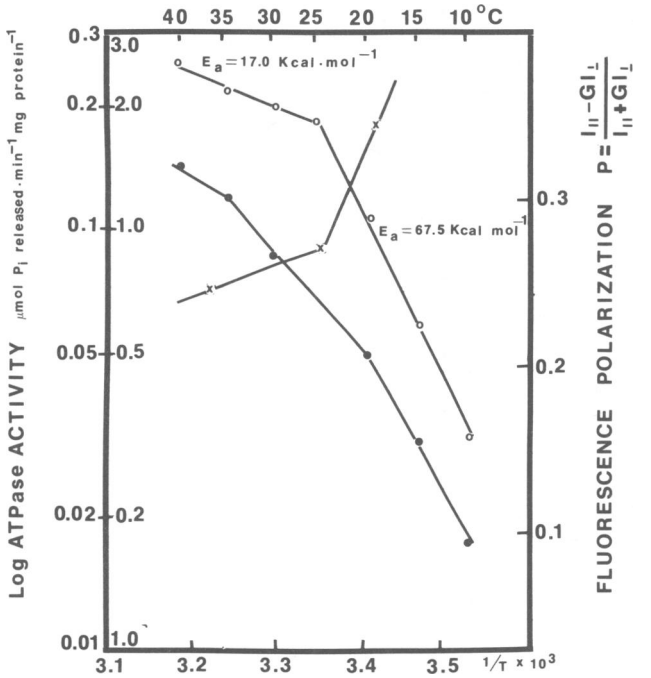

FIGURE 1 Temperature dependence of membrane-bound BF_1 and soluble BF_1-ATPase activity and the fluidity of plasma membrane of *E. coli*. O—O membrane-bound BF_1 ATPase activity; ●—● soluble BF_1 ATPase activity; Δ------Δ membrane fluidity expressed as fluorescence polarization (*P*), which was determined from values of *P* for DPH intercalated in membrane preparations.

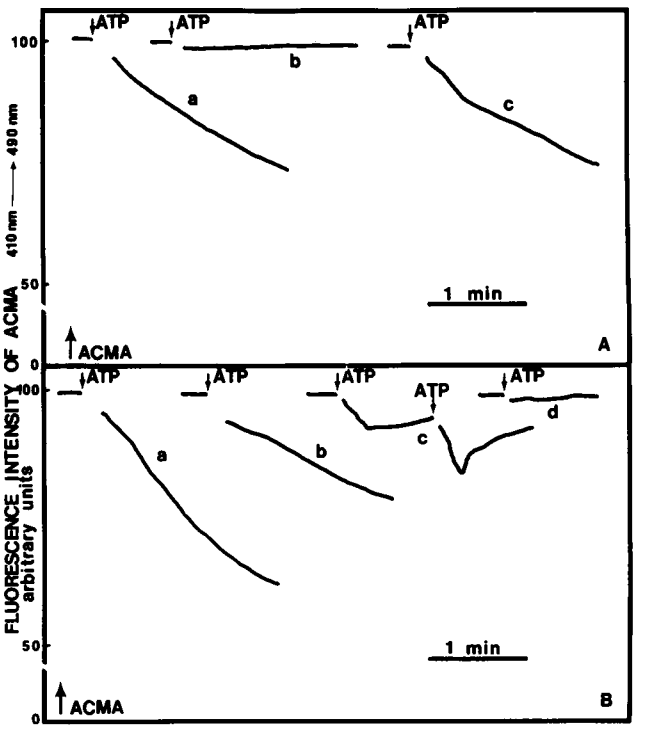

FIGURE 2 Quenching of ACMA fluorescence by *E. coli* plasma membrane. SMP of rat liver mitochondria and cross-hybrid reconstituted membranes. *A*: (*a*) cross-hybrid BF_1–F_0 membrane; (*b*) F_1-depleted SMP; (*c*) native SMP; *B*: (*a*) BF_1–BF_0 reconstituted membrane; (*b*) native *E. coli* plasma membrane; (*c*) F_1–BF_0 cross-hybrid reconstituted membrane; (*d*) BF_1-depleted membrane of *E. coli*.

simplified sonication procedure. SMP and soluble F_1 from rat liver mitochondria were purified by conventional means. Fluorescence polarization analysis was carried out with an RF-502 Correct Spectra Fluorescence Spectrophotometer by using a diphenyl-1,3,5,hexatriene (DPH)-labeled membrane preparation. ATPase activity and other measurements were described previously (4).

RESULTS AND DISCUSSION

Preliminary studies indicated that the *E. coli* plasma membranes obtained showed normal ATPase activity, DCCD-sensitivity, $^{32}P_i$-ATP exchange and ATP-dependent 9-amino-6-chloro-2-methexyacridine (ACMA) fluorescence quenching. Fig. 1 presents some results from studies of the temperature dependence of membrane-bound BF_1-ATPase and soluble BF_1-ATPase activities and of the fluidity of plasma membrane of *E. coli*. An Arrhenuis plot of membrane-bound BF_1-ATPase activity over a temperature range of 10°–40°C shows a single discontinuity at ~ 24°C. The activation energies above and below the transition temperature were 17.0 kcal mol^{-1} and 67.5 kcal mol^{-1}, respectively. The microviscosity values of membranes measured by DPH fluorescence polarization (*P*) at different temperatures also show a sharp break that coincides with the temperature changes in activation energy for membrane-bound BF_1-ATPase. However, an Arrhenuis plot of soluble BF_1-ATPase activity did not show any break at 24°C. From these results and from other work (5) it seems that a lipid phase transition in *E. coli* plasma membrane may occur at ~ 24°C. It is obvious that the activity of membrane-bound BF_1-ATPase activity is modulated by the physical state of the membrane lipid.

Fig. 2 and Table I show some results from cross-hybrid reconstitution of F_1 and F_0 of *E. coli* plasma membrane and rat liver mitochondria. These experiments clearly demonstrate that ATPase (F_1 and BF_1) and coupling membranes from rat liver mitochondria and *E. coli* are completely interchangeable. The resultant cross-hybrid reconstituted BF_1–F_0 membrane and F_1–BF_0 membrane demonstrated restored ATPase activity and DCCD sensitivity as well as ATP-dependent ACMA fluorescence quenching response, although in the case of F_1–BF_0 membrane the ACMA fluorescence quenching was brief: < 1 min. A second addition of ATP could induce the same pattern of ACMA fluorescence quenching. The mechanism of this phenomenon is not yet clear. It may be postulated that the purified F_1 of rat liver mitochondria could not completely block the H^+-channel of BF_0 from *E. coli* plasma membrane. The general, but incomplete, homology in amino acid sequences of DCCD-binding protein of F_0 from *E. coli* and beef heart mitochondria (6) as well as further comparison of the structure of F_1

TABLE I

CROSS-HYBRID RECONSTITUTION OF F_1 AND F_0 OF *E. COLI* PLASMA MEMBRANE AND RAT LIVER MITOCHONDRIA

Materials	ATPase activity (nmol P_i released · min^{-1} · mg $protein^{-1}$)					
	Before reconstitution			After reconstitution		
	−DCCD	+DCCD*	DCCD-sensitive ATPase activity‡	−DCCD	+DCCD*	DCCD-sensitive ATPase activity‡
	BF_1-depleted membrane			F_1-BF_0 cross-hybrid membrane		
E. coli plasma membranes	60	23	37	560	220	340
	60	23	37	760	370	390
	50	23	27	370	130	240
	15	0	0	310	114	186
	F_1-depleted membrane			BF_1-F_0 cross-hybrid membrane		
Rat liver mitochondria	80	0	80	500	200	300
	310	19	291	620	110	510

*DCCD concentration was 0.12 μm.

‡DCCD-sensitive ATPase activity = (−DCCD) − (+DCCD).

between these two species may provide a framework for understanding this phenomenon.

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ASSOCIATION OF THE BOVINE CARDIAC MITOCHONDRIAL ATPase WITH PHOSPHOLIPIDS

Reconstitution and Phospholipid Exchange Studies

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The interaction of various diacyl phospholipids with a highly purified oligomycin-sensitive adenosine triphosphatase (ATPase) preparation (1) from bovine cardiac mitochondria has been examined. Because the phospholipid composition of submitochondrial particles is composed almost exclusively of acidic and neutral phospholipids (2, 3), the effects of these two types of phospholipids on the lipid-depleted mitochondrial ATPase were investigated (Table I). Acidic diacyl phospholipids increased the ATPase specific activity from two- to six-fold, whereas neutral diacyl phospholipids stimulated the ATPase specific activity from zero- to two-fold. Studies with both synthetic and naturally occurring diacyl phospholipids indicated that both the phospholipid fatty acyl side-chain region and the headgroup region affected ATPase activity (Table I). The following order of effectiveness was noted for diacyl phospholipid ATPase activators: asolectin > dioleoylphosphatidylglycerol (DOPG) > dimyristoylphosphatidylglycerol (DMPG) > phosphatidylserine (PS) > dioleoylphosphatidic acid (DOPA) > phosphatidylethanolamine (PE) > dioleoylphosphatidylcholine (DOPC) > dimyristoylphosphatidylcholine (DMPC). Two conclusions were drawn from these findings: (*a*) phospholipids containing fatty acyl side-chains with 18 carbon atoms activate the ATPase better than those containing 14 carbon atoms at 30°C; and (*b*) acidic diacyl phospholipids reactivate the highly purified ATPase better than neutral diacyl phospholipids.

The observations concerning the influence of acyl group composition on the activity of the highly purified ATPase are in agreement with earlier studies carried out with considerably less pure ATPase preparations (4). However, the precise structural characteristics of the fatty acyl chain (e.g. length vs. surface area/mol) which affect the ATPase remain to be clarified. On the other hand, the effect of the diacyl phospholipid headgroup region on the ATPase specific activity appears to be directly related to the ionic charge of the headgroup region. Cunningham and Sinthusek (5) have demonstrated that it is the net negative charge rather than any unique headgroup structure that is important for reactivation of the lipid-depleted ATPase. The relative levels of ATPase specific activity obtained in the present study (Table I) with DOPG, DOPA, and DOPC are very similar to those reported by Cunningham and coworkers (5, 6) with a less pure ATPase preparation and PG, PA, and PC of different fatty acyl chain compositions. However, the absolute levels of specific activity obtained in the present study (Table I) are ~ 10-fold

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