FUNCTION OF ENERGY-DEPENDENT TRANSHYDROGENASE IN ESCHERICHIA COLI

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SUMMARY

The activity of the energy-dependent transhydrogenase of membrane particles of E. coli varied markedly with growth conditions The activity of the enzyme was not related to the efficiency of oxidative phosphorylation. The enzyme was not subject to catabolite repression but was repressed by mixtures of amino acids. It is suggested that the transhydrogenase has a role in generating NADPH for biosynthesis.

INTRODUCTION

The exact role of the energy-dependent transhydrogenase of Escherichia coli (1-6) and other cells is unknown. Although it could act in vivo as a source of NADPH, direct evidence in support of this hypothesis is lacking, and a function as a proton pump has been suggested (7).

There is a close relationship between the enzyme and oxidative phosphorylation in mitochondria since both processes compete for high energy intermediates (8). Thus, in E. coli also, a change in one activity might be expected to affect the other. Now, Hempfling (9) observed that the efficiency of oxidative phosphorylation in E. coli varied with growth conditions. In log phase cells grown on glucose it was low (P:O <0.4) but it was much higher in stationary phase cells (P:O >3). Cells grown on succinate showed maximal P:O values of 1.9 during the log phase. These results indicated

that ATP formation by oxidative phosphorylation was subject to catabolite repression. In line with this, the effect of glucose was abolished by inclusion of 2.5mM cAMP in the growth medium (10).

We have investigated the effect of growth conditions on the energy-dependent transhydrogenase of E. coli to see if variation in the efficiency of oxidative phosphorylation affected this enzyme. No clear relationship between the two processes was observed, and the transhydrogenase was not subject to catabolite repression. Moreover, it was repressed by growth in the presence of amino acids suggesting that it had a role in providing NADPH for biosynthesis.

METHODS

E. coli NRC-482 was grown on a minimal salts medium with 0.4% glucose as previously described (11) except that in some experiments 2% vitamin-free casein hydrolysate (Nutritional Biochemicals) or L-amino acids (each at a final concentration of 0.1%) were present. For growth on a minimal salts medium containing 0.8% disodium succinate 0.1% ammonium sulfate was added also, and the pH of the medium was maintained at pH 7.2-7.4 during growth by additions of 5 N HCl. Except where noted, the above media contained 12μM ferric citrate. The complex glucose medium was 3% trypticase-soy medium (BBL). The cells were grown at 37° with vigorous aeration from a sparger except for growth on complex media where a reciprocating water bath shaker was used. The cells were harvested mid to late log or in the stationary phase of growth and were washed once with 50mM Tris sulfate buffer, pH 7.8, containing 10mM $MgCl_2$ (TM buffer). The cells were suspended in TM buffer (1 g wet weight per 10 ml buffer) and disrupted in a French press (Aminco) at 20,000 psi. The suspension was centrifuged at 17,000 x g for 10 min to remove whole cells and large cell fragments. The respiratory particles were obtained by cen-

trifuging the supernatant from the above step at 120,000 x g for 2 hours. The resultant pellet was suspended in TM buffer containing 1% bovine serum albumin at a concentration of 10-15 mg particle protein per ml.

Transhydrogenase activity was measured by a modification of the procedure of Fisher and Sanadi (5). 1 ml TM buffer containing 0.1% bovine serum albumin, 0.1mM dithiothreitol and 0.7 M sucrose was preincubated with 50 µl particle suspension in a cuvette at 37° for 5 min. The cuvette was then transferred to a Coleman 124 spectrophotometer equipped with recorder and the absorbance followed at 340 nm after addition of 5 µl ethanol, 50 µl yeast alcohol dehydrogenase (4 mg per ml; Calbiochem) and 25 µl 3mM NAD. After 0.66 min 50 µl 16.3mM NADP was added and the reduction of NADP measured ("aerobic-driven transhydrogenase"). When the oxygen in the cuvette was exhausted the reduction of NADP abruptly changed to a lower rate ("energy-independent transhydrogenase"). When this rate had been established 10 µl 60mM ATP was added and the new rate of NADP reduction measured ("ATP-driven transhydrogenase"). The rates of the two energy-driven transhydrogenase activities were corrected for the contribution made by the energy-independent transhydrogenase. NADH oxidase activity was calculated from the time taken to deplete oxygen in the cuvette. NADPH:O ratios were calculated from the amount of NADPH formed during the aerobic phase.

ATPase activity was measured at 37° in the presence of 5mM $CaCl_2$ as described before (12). P:O ratios given by respiratory particles were determined at 30° by the method of Butlin et al. (13) except that oxygen uptake was measured polarographically. Cytochromes were quantitated using the extinction coefficients given by Jones and Redfearn (14).

TABLE 1. Effect of growth conditions on energy-dependent transhydrogenase and other activities of respiratory particles

Medium	Additions	Harvested	ATPase	NADH oxidase	Cytochrome b_1	Cytochrome a_2	Transhydrogenase Aerobic	Transhydrogenase ATP	Transhydrogenase NADPH:O
Salts-succinate	No Iron	L	839	1160	0.23	0.08	21	33	0.07
	--	L	773	1230	0.49	0.16	33	55	0.04
Complex glucose	No Iron	L	397	1330	0.57	0.43	0	11	0
	No Iron	S	368	1030	0.85	0.56	0	17	0
Salts-glucose	No Iron	L	--	1730	0.27	0	84	60	0.15
	No Iron	S	--	1890	0.21	0.05	58	67	0.13
	--	L	406	1770	0.28	0.06	89	75	0.15
	--	S	476	1880	0.34	0.07	65	69	0.10
	2.5mM cAMP	L	783	1380	0.33	0.04	49	68	0.09
Salts-glucose (anaerobic)	--	L	686	--	--	--	61	80	0.14

L, logarithmic phase; S, stationary phase. Cytochrome concentration is expressed as nmoles/mg protein, ATPase and transhydrogenase activities as nmoles/min/mg protein, and NADH oxidase as nmoles/min/nmole cytochrome b_1.

RESULTS AND DISCUSSION

Table I shows the effect of growth conditions on the energy-dependent transhydrogenase activities of respiratory particles from E. coli. Cytochrome content, and NADH oxidase and ATPase activities are included since the aerobic-driven transhydrogenase derives its energy from oxidation of NADH via the cytochrome chain, and the ATPase has been implicated in the ATP-driven reaction (6). Both transhydrogenase levels (and cytochrome a_2) varied markedly with growth conditions and to a greater extent than did cytochrome b_1, ATPase and NADH oxidase. Highest transhydrogenase activities occurred during the log phase of growth on glucose and were somewhat lower when the cells had entered the stationary phase. Enrichment of the growth medium with 12μM ferric citrate did not significantly affect the transhydrogenase activities although it increased the cytochrome content. Addition of cAMP or growth on glucose under anaerobic conditions also did not greatly affect the transhydrogenase activities. When the cells were grown on succinate the energy-dependent transhydrogenase activities were lower.

Since these results suggested that there might be a reciprocal relationship between the aerobic-driven transhydrogenase and ATP-formation (P:O value) as predicted by Hempfling's results, we measured oxidative phosphorylation in our respiratory particle preparations (Table 2). The P:O values were low, probably due to lack of coupling factors (2), but could be measured reproducibly by the technique employed. No relationship was found between the aerobic-driven transhydrogenase activity and the P:O values. Thus, although the situation could be different in whole cells, the variation in the transhydrogenase activity with growth conditions does not seem to be due to variation in the ability of

TABLE 2. Effect of growth conditions on oxidative phosphorylation performed by respiratory particles

Medium	Harvested	P:O		
		NADH	D-Lactate	Succinate
Salts-glucose	L	0.082	0.082	0.076
	S	0.071	0.062	0.052
Salts-succinate	L	0.075	0.110	0.078
Complex glucose	L	0.091	0.067	0.044
	S	0.095	0.041	0.045

L, logarithmic phase; S, stationary phase

the various preparations to carry out oxidative phosphorylation, or to differences in ATPase, NADH oxidase and cytochrome b_1 levels.

With respiratory particles prepared from cells grown on trypticase-soy medium (Table 1) or on casein hydrolysate, with or without glucose (Table 3), the aerobic-driven transhydrogenase could not be detected and the ATP-driven reaction was much less active. Growth on glucose in the presence of selected groups of amino acids or single amino acids indicated that this effect could not be attributed to any one amino acid. These data suggest that the energy-dependent transhydrogenase has a role in supplying NADPH for the biosynthesis of amino acids (and other intermediates), and that formation of this enzyme is repressed when the preformed end-product is supplied.

Further support for the function of the transhydrogenase in anabolism was obtained using three catabolite derepressed mutants (cat 5,6 and 11) derived from E. coli B. The energy-dependent transhydrogenase and ATPase activities of the mutants did not differ significantly from those of the parent strain.

TABLE 3. Effect of growth in the presence of amino acids on the energy-dependent transhydrogenase of respiratory particles

Medium	Additions	Transhydrogenase			ATPase
		Aerobic	ATP	NADPH:O	
Salts	Casein hydrolysate	0	18	0	668
Salts-glucose	--	89	75	0.15	406
	Casein hydrolysate	0	11	0	569
	His,ser,gly	16	33	0.05	670
	Tyr,phe	43	63	0.09	666
	Lys,met,pro,arg,glu	0	21	0	644
	Ileu,Val,leu,thr	0	16	0	625
	Ileu	23	25	0.05	633
	Val	19	21	0.05	539
	Leu	13	17	0.03	642
	Thr	23	25	0.06	622
	Met	23	43	0.06	

Enzyme activities are expressed as nmoles/min/mg protein

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