EVIDENCE FOR OXIDATIVE PHOSPHORYLATION IN STREPTOCOCCUS FAECALIS[1]

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There exists little or no evidence for oxidative phosphorylation in non-cytochrome-containing microorganisms. Theoretically, however, an organism such as the lactic acid bacterium, Streptococcus faecalis, while lacking the hematin carriers (Dolin, 1953), could catalyze the formation of ATP on oxidation of DPNH. Potential sites of oxidative phosphorylation would be at the DPNH/flavin level and during the oxidation of reduced naphthoquinone since this species possesses both flavins and naphthoquinones (Baum and Dolin, 1963). Dolin (1955) in a previous study using the sensitive firefly luminescent assay system failed to detect oxidative phosphorylation on DPNH oxidation by anaerobically-grown Streptococcus faecalis strain 10C1. The present communication presents evidence for ATP formation during the oxidation of DPNH by cell-free extracts of this organism grown under aerobic conditions.

MATERIALS AND METHODS

Streptococcus faecalis strain 10C1 was cultured under anaerobic or aerobic conditions as previously described (Jacobs and VanDemark, 1959). Lysozyme and French pressure cell extracts were prepared according to the procedure of Moore and O'Kane (1963). The protein level of cell extracts was estimated by the trichloroacetic acid method of Stadtman et al. (1951), using crystalline bovine serum albumin as a standard. The oxidation of

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DPNH was followed spectrophotometrically as the decrease in absorbancy at 340 mμ. Oxidative phosphorylation was assayed using the spectrophotometric method of Pinchot (1953) and as the rate of ^{32}P incorporation during the oxidation of DPNH. In the latter spectrophotometric method the hexokinase-glucose trap system was employed with one cuvette containing the complete reaction mixture listed in the protocol of Table 1, including TPN, and a second cuvette containing all components of the reaction mixture except TPN. The change in absorption at 340 mμ in the latter cuvette is a measure of DPNH oxidation and the difference in the absorption change between the two cuvettes a measure of TPNH formation, with the P/O ratio calculated from the μmoles of TPNH accumulating per μmole of DPNH oxidized.

For the rate of ^{32}P incorporation during the oxidation of DPNH, the oxidation of DPNH was followed spectrophotometrically, with the reaction mixture listed in Table 3. The reaction was stopped by the addition of 0.1 ml cold 4.4 N perchloric acid. Nucleotides were adsorbed on charcoal by the addition of Norit A in 50% ethanol according to the method of Crane and Lipmann (1953). The charcoal was collected on a membrane filter (0.65 μ pore size), washed twice with 3.0 ml distilled H_2O and twice with 4.0 ml 0.2 M phosphate buffer, pH 7.2. The filters were dried and then activity measured with a thin window gas flow counter. Samples were counted to 3000 counts with $\sigma = 55$.

All chemicals were commercial preparations; DPNH, TPN, AMP, ADP and antimycin A were purchased from Calbiochem, hexokinase (Type II) and G-6-P dehydrogenase (Type V) from Sigma Chemical Co., and Norit A from Pfanstiehl Chemical Co.

RESULTS AND DISCUSSION

Evidence for oxidative phosphorylation in the cell-free preparation of aerobically-grown Streptococcus faecalis using the spectrophotometric method of Pinchot (1953) is shown in Table 1.

TABLE 1

Phosphorylation coupled to DPNH oxidation by S. faecalis extracts

Reaction system[1/]	Cells grown	DPNH oxidized	TPNH formed	P/O
		mμmoles/min/mg protein		
1. Complete	aerobic	38.6	9.1	0.24
2. Less ADP	aerobic	41.0	0.0	0.00
3. Less ADP, plus AMP	aerobic	24.5	1.7	0.07
4. Complete	anaerobic	20.0	0.0	0.00

1/ The concentration of components of the complete reaction system/ml was:

glycylglycine (pH 7.2)	13 mM	hexokinase	5 units
K phosphate (pH 7.2)	3 mM	G-6-P dehydrogenase	3000 units
$MgCl_2$	3 mM	bovine serum albumin	0.03 mg.
KF	3 mM	S. faecalis extract	0.15 mg.
glucose	10 mM		
ADP or AMP	0.3 mM		
DPNH	0.15mM		
± TPN	0.15mM		

With the use of crude extracts the possibility exists of the conversion of 2 molecules of ADP to ATP and AMP via a myokinase reaction which would result in the observed TPN reduction in the present assay system. Thus, as shown in Table 1, when AMP was substituted for ADP there occurs a low but consistently repeatable level of phosphorylation coupled with the oxidation of DPNH, eliminating the possibility that the observed TPN reduction was due to a myokinase reaction.

The occurrence of phosphorylation during DPNH oxidation by aerobic but not anaerobically-grown cells would seem to reflect a difference in the respiration route of these two types of cells. Dolin (1955) demonstrated DPNH oxidation in anaerobically-grown S. faecalis involves a DPNH oxidase which catalyzes the two-electron reduction of O_2 to H_2O_2, with the overall oxidation being a four-electron reduction of O_2 due to the complementary action of DPNH peroxidase. In contrast Hoskins et al.

(1962) have demonstrated that the purified DPNH oxidase of aerobically-grown S. faecalis catalyzes the four-electron reduction of oxygen to water without the obligatory participation of a DPNH peroxidase. Thus the present studies complement these prior enzymatic investigations by indicating further differences in the pathway of DPNH oxidation by S. faecalis induced by aerobiosis.

As shown in Table 2, various known uncouplers of oxidative phosphorylation diminished or prevented phosphorylation during DPNH oxidation by S. faecalis. However, as might be predicted for a respiratory chain not containing cytochrome oxidase NaN_3 or KCN did not prevent oxidative phosphorylation.

The sensitivity of DPNH oxidation and the coupled phosphorylation

TABLE 2

Inhibitors of oxidative phosphorylation by extracts of aerobically grown S. faecalis[1/]

Reagent	Concentration	DPNH oxidized	TPNH formed	P/O
	mM	mμmoles/min/mg/protein		
1. None	-	30.8	6.8	0.22
2. 2,4-DNP[2/]	0.08	34.3	0.0	0.00
3. Quinacrine.HCl	0.02	30.8	1.7	0.06
4. Methylene blue	0.08	6.8	0.0	0.00
5. Antimycin A	0.08	10.2	0.9	0.08
6. KCN	0.4	39.3	6.8	0.17
7. NaN_3	0.1	20.4	3.4	0.17
8. Na_2AsO_3	10.0	37.5	0.0	0.00

1/ Protocol as in Table 1. The complete system less DPNH, was preincubated 5 min. with each system, and the reaction started by DPNH addition.

2/ 2,4-Dinitrophenol

in this cytochrome-less microorganism to antimycin A would imply that the site of antimycin A inhibition does not directly involve the cytochromes. Furthermore, since Baum and Dolin (1963) have demonstrated the presence of a naphthoquinone in this organism, the site of antimycin A inhibition may be at this respiratory site.

TABLE 3

Incorporation of ^{32}P during DPNH oxidation

Reaction system[1/]	DPNH oxidized	P_i esterified	P/O
	μmoles/min/mg protein		
1. Complete	2.00	0.59	0.29
2. Less ADP	0.80	0.01	0.01

1/ Cuvettes contained 20 μmoles glycylglycine buffer, pH 7.2, 3 μmoles $MgCl_2$, 3 μmoles KF, 0.03 mg serum albumin, 20 μmoles K phosphate (containing 540 c.p.m. ^{32}P per μmole carrier phosphate), with or without 0.5 μmoles ADP, 0.3 μmoles DPNH and approximately 2 mg protein extract of aerobically-grown cells. The values have been corrected for endogenous ^{32}P esterification in a reaction mixture containing all components less DPNH.

Table 3 illustrates evidence for oxidative phosphorylation by cell-free preparations of aerobically-grown S. faecalis measured as the incorporation of ^{32}P into ATP during the oxidation of DPNH. Using this assay system P/O ratios were obtained analogous to that found with the hexokinase-glucose trap system. With the hexokinase-glucose system the possibility exists that the observed phosphorylation is actually due to substrate phosphorylation resulting from the fermentation of the added glucose. Incorporation of ^{32}P into ATP during the oxidation of DPNH in the absence of the added glucose-hexokinase trap system would appear to eliminate this possibility.

Conceivably in this respiratory chain involving flavins and naphthoquinones one might predict a theoretical P/O ratio approaching 2 on the

oxidation of DPNH. However, as is characteristic of many bacterial preparations (Pinchot, 1953; Nossal et al., 1956; and Tissières et al., 1957), the P/O ratio observed on DPNH oxidation by S. faecalis is relatively low.

The actual significance of oxidative phosphorylation in this species is questionable since aeration results in an increase in cell yield of approximately one-third that obtainable under anaerobic conditions (Seeley and VanDemark, 1951). Since pyruvate is no longer serving as a terminal hydrogen acceptor under aerobic conditions, this increase in growth could be due to pyruvate dismutation, to form acetyl-CoA thereby yielding the cell additional energy.

Further studies of oxidative phosphorylation by this organism seem warranted since the mechanism might be more readily clarified in this abbreviated electron-transport chain uncomplicated by hematin respiratory carriers.

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