INTERCHANGEABILITY OF COUPLING FACTORS FROM BACTERIAL AND MAMMALIAN ORIGIN

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SUMMARY

Bacterial and mammalian preparations capable of oxidative phosphorylation can be separated into a particulate fraction containing the electron transport chains and a soluble fraction containing coupling proteins necessary for restoration of phosphorylation. The addition of coupling factors of bacterial origin to sub-mitochondrial particles was found to replace the requirement for mammalian coupling factors for restoration of phosphorylation. Coupling factors obtained from light beef heart mitochondria were found to restore phosphorylation to the electron transport particles of Mycobacterium phlei. The level of phosphorylation restored by the heterologus coupling factors was found to be lower than that restored by the native coupling factor; however, the phosphorylation restored by either coupling factor was found to be sensitive to uncoupling agents.

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INTRODUCTION

Systems capable of oxidative phosphorylation can be dissociated into a structurally intact component capable of only oxidation and into soluble protein components required for restoration of phosphorylation. The soluble protein components required for restoration of phosphorylation are referred to as coupling factors and have been found in bacteria and mammalian (1-11) preparations capable of oxidative phosphorylation and in plant chloroplasts (12) capable of photosynthetic phosphorylation. The phosphorylation restored by the addition of coupling factors to the electron transport particles, like the unsolved system, was shown to be sensitive to uncoupling agents and was abolished by anaerobiosis or respiratory inhibitors.

Previous studies with bacterial systems have shown that the soluble coupling factors obtained from different microorganisms could be substituted for the native coupling factor for restoration of phosphorylation (3). Schatz et al (13) have shown that a preparation from bakers yeast which had ATPase activity was capable of restoring phosphorylation to submitochondrial beef heart particles. It was thus of interest to determine whether the coupling factors from mammalian mitochondria could restore phosphorylation to the M. phlei electron transport particles and the effects of the M. phlei coupling factors of beef heart submitochondrial particles.

METHODS AND MATERIALS

M. phlei ATCC 354 was grown and harvested by procedure previously described (3). Disruption of whole cells, isolation and washing of the electron transport particles was the same as that described earlier (3). In order to remove the bound coupling factor (BCF_4) the particles were suspended in 0.25M sucrose (3.0 mg/ml) in the absence of any salt and centrifuged 140,000 x g for 60 minutes. The precipitate containing the bacterial electron transport particles free of BCF_4 are referred to as "sucrose particles." Coupling factor BCF_4 was purified from the sucrose supernatant by density gradient centrifugation (11).

Beef heart mitochondria were prepared according to the method of Green and Ziegler (15). Heavy mitochondria obtained by this procedure (15) were used for the preparation of the electron transport particles according to Linnane and Titchener (5). The supernatant obtained following sonication (2 minutes) and centrifugation at 105, 000 x g for 60 minutes of both heavy or light mitochondria was tested for the coupling factor activity.

Oxygen uptake was measured by the manometric technique with a Gilson respirometer at 30°. Inorganic phosphate esterification was measured by the disappearance of orthophosphate from the reaction mixture according to Fiske and SubbaRow (16). Protein content was determined by the trubidimetric method of Stadtman, Novelli and Lipmann (17).

RESULTS AND DISCUSSION

M. phlei particles contain a bound coupling factor (BCF_4). This coupling factor was removed from this particle by washing the particles in 0.25M sucrose in the absence of salt. This procedure resulted in the separation of the electron transport particles (sucrose particles) from the coupling factor which remained in the supernatant (BCF_4). The removal of BCF_4 from the electron transport particles resulted in a loss of phosphorylation without any effect on the level of oxidation (Table 1). The addition of BCF_4 to the electron transport particles resulted in complete restoration of the phosphorylation.

Addition of the mitochondrial supernatant obtained following centrifugation at 105, 000 x g for 60 minutes of sonicated heavy mitochondria to coupling factor-depleted M. phlei particles (sucrose particles) failed to restore phosphorylation. However, the crude supernatant obtained following sonication of light mitochondria was found to restore phosphorylation to the depleted M. phlei sucrose particles, the level of restoration was only 30-50 percent when compared to the level of phosphorylation restored with M. phlei BCF_4 (Table 1).

Submitochondria particles obtained from the heavy layer of beef heart mitochondria, required the addition of the supernatant from sonicated heavy mitochondria for restoration of phosphorylation. Supernatant obtained from the

Table 1

The Effect of Mitochondrial and M. phlei Coupling Factors on the Oxidative Phosphorylation of M. phlei Particles

	O_2 μatoms	P_i μmoles	P/O
Regular particles	10.2	8.8	0.86
Regular particles + gramicidin A	11.6	0.8	0.07
Sucrose washed particles	10.1	1.3	0.13
Sucrose washed particles + gramicidin A	10.3	0.4	0.04
Sucrose washed particles + BCF_4	9.9	8.2	0.83
Sucrose washed particles + BCF_4 + gramicidin A	10.0	0.5	0.05
Sucrose washed particles + heavy mitochondria supernatant	9.6	1.6	0.16
Sucrose washed particles + light mitochondria supernatant	9.8	3.7	0.38
Sucrose washed particles + light mitochondria supernatant + gramicidin A	10.1	0.0	0.00

The system consisted of 100 μmoles of HEPES-KOH buffer pH 7.4, 50 μmoles of glucose, 15 μmoles of $MgCl_2$, 15 μmoles of orthophosphate, 3 mg of yeast hexokinase, 25 μmoles KF, 5 μmoles of ADP, 20 μmoles of hydrazine, 0.5 mg crystalline alcohol dehydrogenase, 0.5 Umole NAD^+, electron transport particles, supernatant as indicated and water to a final volume of 2.0 ml. The reaction was started by the addition of 100 μmoles ethanol from the side arm of the vessel. Protein content of M. phlei particles was 3.0 mg, BCF_4, heavy or light mitochondrial supernatant 0.5mg. The correct ration of gramicidin A was 10 μgrams.

light layer of the mitochondria also restored phosphorylation to the submitochondrial fraction; however, only to the level of 40 to 60 percent as compared to the supernatant obtained from the heavy layer of the mitochondria. Similar results were obtained with the M. phlei coupling factor BCF_4 when added to the submitochondrial particles (Table 2). The addition of the bacterial coupling factor BCF_4 was found to have no effect on oxidation.

Although the ability to restore phosphorylation with the heterologous coupling factor was found to be linear at low concentration of protein, high concentrations of either coupling factor with the heterologous electron transport particles resulted in an inhibition or loss in ability to restore phosphorylation. Of particular interest was the finding that the phosphorylation restored by

Table 2

The Effect of Mitochondrial and M. phlei Coupling Factors on the Oxidative Phosphorylation of Submitochondrial Particles

	O_2 μatoms	P_i μmoles	P/O
Submitochondrial Particles	10.9	1.8	0.16
Submitochondrial Particles + DNP (7×10^{-4}M)	11.2	1.1	0.10
Submitochondrial Particles + heavy mitochondria supernatant	10.6	9.8	0.92
Submitochondrial Particles + heavy mitochondria supernatant + DNP (7×10^{-4}M)	10.9	4.1	0.37
Submitochondrial Particles + light mitochondria supernatant	10.6	4.2	0.40
Submitochondrial Particles + M. phlei BCF_4	10.3	3.6	0.35
Submitochondrial Particles + M. phlei BCF_4 + DNP (7×10^{-4}M)	10.3	2.3	0.22
Submitochondrial Particles + M. phlei BCF_4 + gramicidin A	10.9	0.5	0.05

Conditions were similar to those described in Table 1. 3.0 mg protein of submitochondrial particles were used.

either BCF_4 or the supernatant from heavy mitochondria and the bacterial electron transport particles was completely uncoupled by Gramicidin A at low concentration (10 μg). Gramicidin A has been found to be the most effective uncoupling agent with the M. phlei system (4). Both DNP (7×10^{-4}M) and gramicidin A were found to uncouple the phosphorylation restored by the addition of the mammalian or bacterial coupling factor to submitochondrial particles; however, the degree of uncoupling by DNP with the bacterial coupling factor was lower than that observed with the mammalian coupling factor. Nevertheless, with gramicidin A complete uncoupling was observed with either coupling factor.

It would appear that the electron transport particles yield the highest phosphorylation levels when incubated together with its native coupling protein. However, exchange of coupling factors of either bacterial and mammalian origin with the heterologous electron transport particles resulted in a partial restoration of phosphorylation. One possible explanation for a partial restoration is that the coupling factor plays a dual role and that only one role is fulfilled. One

function may be catalytic and the other may be structural as suggested by Penefsky (10).

In conclusion, it is of interest that coupling factors from M. phlei were capable of restoring phosphorylation in coupling factor-depleted beef heart sub-mitochondria. A soluble factor from light beef mitochondria restored phosphorylation to the coupling factor-depleted M. phlei particles. These findings are consistent with the earlier observations of the exchange of coupling factors between different bacterial systems (3).

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