A COUPLING FACTOR FROM *RHODOSPIRILLUM RUBRUM* CHROMATOPHORES

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1. Introduction

A coupling factor from the photophosphorylation system of *Rhodopseudomonas capsulata* has recently been isolated [1]. By using a slightly modified procedure, as compared to that used on *Rps capsulata*, on *Rhodospirillum rubrum* chromatophores, a protein factor which seems to be necessary for photophosphorylation and Mg-ATPase activities, has been solubilized. The factor is supposed to be involved in the coupling mechanism.

2. Materials and methods

R. rubrum cells (strain S1) were grown and harvested and chromatophores prepared according to methods described earlier [2]. Photophosphorylation [3] and ATPase [4, 5] activities were assayed by standard methods. Protein was determined by the Biuret method. The protein factor was solubilized and reconstituted as is shown in fig. 1. Further details are given under the tables.

3. Results and discussion

After sonication in the cold and separation of supernatant, photophosphorylation and Mg-ATPase activities of the particles were depressed to 15–50% of the original values. Restoration of photophosphorylation and Mg-ATPase was obtained after incubation of treated particles together with supernatant and 10 mM $MgCl_2$ for 1 hr at room temp. The parallelism observed between photophosphorylation and Mg-ATPase activities, as induced by the sonication and reconstitution treatments (table 1), seems to indicate a coupling factor effect at the energy transfer level.

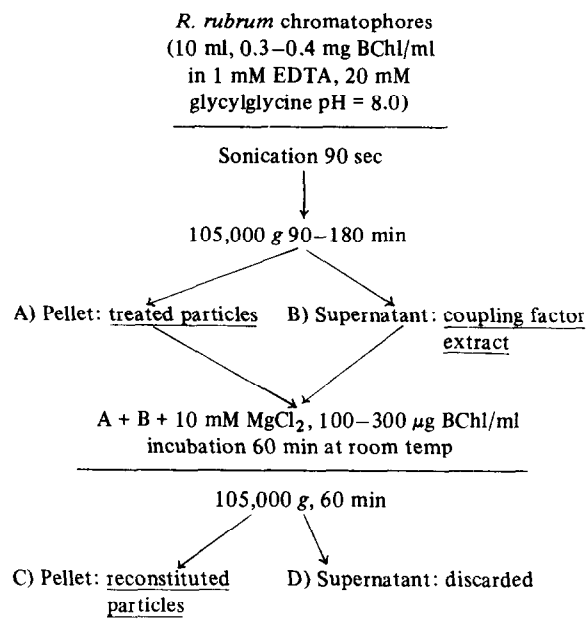

Fig. 1. Procedure for solubilization and reconstitution of coupling factor.

The degree of restoration was clearly a function of the amount of supernatant added. With 2 equivalents of supernatant, a photophosphorylation value exceeding that of the untreated particles was obtained (table 2).

Table 1
Effects of removal and restoration of coupling factor on photophosphorylation and ATPase activities.

Treatment of chromatophores	Photophosphorylation activity (%)	MgATPase activity (%)
(1) None	100	100
(2) Sonication	24	26
(3) As (2), but with an equivalent amount of supernatant and 10 mM $MgCl_2$ added	62	74

Reaction medium for photophosphorylation: 0.1 M glycylglycine pH 7.4, 3×10^{-4} M ATP, 3×10^{-4} M Na_2HPO_4 containing ^{32}P, 10^{-2} M $MgCl_2$, 3×10^{-4} M Na succinate, excess hexokinase, chromatophores to a final conc. of $A_{800} = 0.2$ in a total vol of 3 ml. 6 min exp. at 30°. Untreated particles: 58 μmoles ATP formed/hr × A_{800}. Reaction medium for ATPase: 5×10^{-2} M Tris-HCl pH 7.5, 5×10^{-3} M $MgCl_2$, 5×10^{-3} M ATP, chromatophores in a final conc. of $A_{800} = 1.0$. Total vol 1 ml. 10 min exp. at 30°. Untreated particles: 4.8 μmoles ATP hydrolyzed/hr × A_{800}.

The protein nature of the factor responsible for the reported changes was shown by its sensitivity to trypsin treatment (table 3).

Effects of the protein factor on cytochrome *b* reduction and carotenoid band shift as induced by ATP or PP_i in the dark, as well as on electron transport control in this system have now also been demonstrated [6]. The carotenoid data further support the concept that the coupling factor effect is at the energy transfer level.

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Table 2
Restoration of photophosphorylation as a function of added amount of supernatant.

Supernatant added (ml)	(equivalents)	Phorophosphorylation (% of untreated)
0	0	39
0.5	0.25	57
1.0	0.5	74
2.0	1.0	85
4.0	2.0	112

Reaction medium for photophosphorylation: as in table 1. Untreated particles: 46 μmoles ATP formed/hr × A_{800}.

Table 3
Specific effect of trypsin on coupling factor.

Treatment	Photophosphorylation activity (%)
(1) Untreated	100
(2) Sonicated	50
(3) Reconstituted	94
(4) As (3) but trypsin treated supernatant	52
(5) As (4) but with soybean inhibitor	90

Reaction medium for photophosphorylation: as in table 1. Trypsin treatment: coupling factor supernatant was incubated with 320 μg trypsin/mg protein for 10 min at 30°. Digestion was stopped by addition of soybean inhibitor to a final conc. of 1290 μg/mg protein. Control (5): supernatant incubated with trypsin + soybean inhibitor.

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