INORGANIC PYROPHOSPHATE AS AN ENERGY DONOR IN PHOTOSYNTHETIC AND RESPIRATORY ELECTRON TRANSPORT PHOSPHORYLATION SYSTEMS

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Inorganic pyrophosphate (PPi) is the end product of photophosphorylation in chromatophores from Rhodospirillum rubrum in the absence of added adenine nucleotide (H. Baltscheffsky et al., 1966). This formation of PPi, which is insensitive to oligomycin, has been proposed to occur in a sequence of reversible reaction steps (H. Baltscheffsky and von Stedingk, 1966).

It has earlier been reported that addition of PPi or ATP to chromatophores causes an energy requiring absorbance change in the Soret region of the spectrum. This change was attributed to reduction of endogenous cytochrome. When induced by PPi it was insensitive to oligomycin, whereas this compound inhibited the ATP-induced absorbance change (M. Baltscheffsky et al., 1966). The main component responsible for the absorbance change has later been identified as b-type cytochrome, which becomes reduced upon addition of PPi or ATP (M. Baltscheffsky, in preparation). The action of PPi on c-type cytochrome as well as on b-type cytochrome, allowing identification of the exact localization of a coupling site in bacterial photophosphorylation, is described in this paper. Also reported here are some PPi-induced cytochrome changes which have for the first time been observed in various respiratory electron transport coupled phosphorylation systems.

MATERIAL AND METHODS

Rhodospirillum rubrum (Strain S-1) was grown in the medium described by Bose et al. (1961) and chromatophore preparations ("chromatophore fragments") were made according to H. Baltscheffsky (1960). The blue-green mutant of Rhodospirillum rubrum (Strain G-9)[x] was grown in a medium consisting of 0.3% yeast extract and 0.2% casamino acids (Difco) in distilled water, and the chromatophores were prepared as above.

Mitochondria from Saccharomyces cerevisiae were prepared according to the method described by Ohnishi and Hagihara (1964). Rat liver mitochondria were prepared by standard methods (homogenization in a Potter-Elvehjem homogenizer, subsequent differential centrifugation, two washings). The medium used throughout contained: 0.225 M mannitol, 0.075 M sucrose, 0.1 mM EDTA and 10 mM Tris, pH 7.4. The absorbance changes have been measured in a double beam spectrophotometer (Chance, 1954) or in an Aminco-Chance Dual-Wavelength Spectrophotometer.

RESULTS AND DISCUSSION

In bacterial photophosphorylation it has been proposed that two coupling sites exist (H. Baltscheffsky et al., 1960), and that one of these is located in the cytochrome region (H. Baltscheffsky et al., in preparation). However, no strong evidence was available about the exact location of this site. Our demonstration that both PPi and ATP caused a reduction of endogenous b-type cytochrome by reversed reactions of photophosphorylation in Rhodospirillum rubrum chromatophores opened a new possibility for the localization of the suggested coupling site in the cytochrome region. It was recalled that Klingenberg (1961) found, in skeletal muscle mitochondria, that addition of ATP induced an oxidation of cytochrome c and a reduction of cytochrome b, which was interpreted as a reversal of oxidative phosphorylation, showing a

[x] The author is indebted to Dr. M. Nishimura for his help in providing cultures of this organism.

cross-over point between cytochrome b and c, applying the cross-over theorem introduced earlier by Chance et al. (1955).

In chromatophores from Rhodospirillum rubrum (Strain S-1) it is extremely difficult to obtain any evidence for the involvement of c-type cytochrome in the reversal of photophosphorylation because of the strong interference by the absorption changes of carotenoids. It was possible to overcome this experimental difficulty by using chromatophores prepared from a carotenoid-deficient blue-green mutant of this organism (Strain G-9). These chromatophores gave a PPi-induced reduction of b-type cytochrome as the chromatophores from Strain S-1, measured at 427 minus 407 mμ and, as is shown in Fig. 1, also enabled us to look at the PPi-induced changes of the α-bands of endogenous cytochromes. Addition of PPi gave a reduction of b-type cytochrome and an oxidation of c-type cytochrome, in other words, there is a cross-over point between these cytochromes in the reversal of bacterial photophosphorylation.

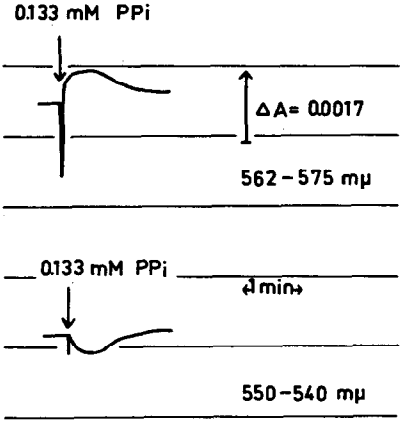


Fig. 1. Spectroscopic changes of endogenous b- and c-type cytochromes in R. rubrum blue-green mutant chromatophores induced by addition of PPi. 0.2 M glycyl-glycine buffer pH 7.5, 3.3 mM $MgCl_2$ and chromatophores equivalent to $O.D._{800}$ = 1.44. Total volume 1.5 ml.

The coupling site thus found can be regarded as analogous to the second coupling site in oxidative phosphorylation, as was suggested by H. Baltscheffsky (1966), and constitutes the first exactly localized phosphorylation site in a photosynthetic electron transport system.

As an extension of the studies on the role of PPi as an energy donor, its possible effect on respiratory systems was investigated, first on mitochondria from Saccharomyces cerevisiae. Fig. 2 snows the reduction of

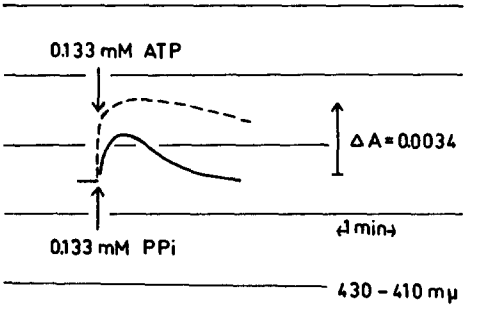


Fig. 2. Reduction of endogenous cytochrome b by PPi (solid line) and ATP (dashed line) in mitochondria from Saccharomyces cerevisiae. 0.2 M glycylglycine buffer pH 7.5, 3.3 mM $MgCl_2$ and mitochondria giving a final concentration of 3.9 mg protein/ml. Partial reduction of the electron transport chain was obtained by addition of 0.67 mM Na_2S. Total volume 1.5 ml.

cytochrome b induced by PPi. The ATP-induced reduction has been included as a comparison. A PPi-induced oxidation of cytochromes c and a was also found, measured at 550 minus 540 mμ and 605 minus 630 mμ, respectively.

In order to explore the possible generality of the PPi-effect in respiratory systems mitochondria from Neurospora crassa[x] and rat liver were tested and were also found to respond by reduction of cytochrome b, in oligomycin insensitive reaction pathways. Fig. 3 shows this reduction in rat liver mitochondria and its insensitivity to oligomycin. The similar but larger changes obtained when ATP was used as energy donor instead of PPi were inhibited by oligomycin, as is shown in Fig. 4.

The present demonstration that PPi can provide the energy for energy-requiring reversed electron transport at the cytochrome level in an oligomycin

[x]The experiments with Neurospora were performed in collaboration with Drs. D.O. Hall and H. Baltscheffsky (in preparation).

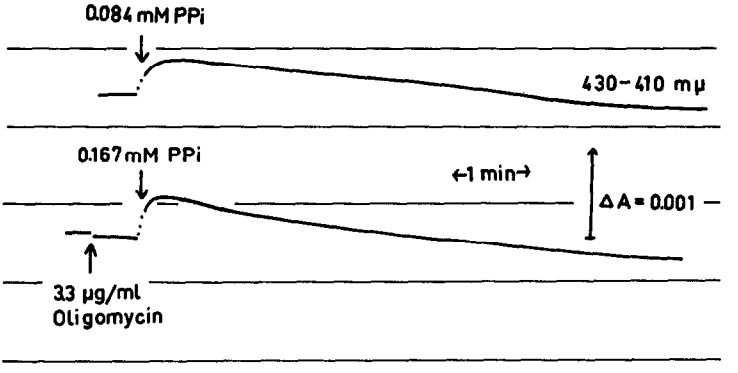


Fig. 3. Reduction of endogenous cytochrome b by PPi in rat liver mitochondria. 0.225 M mannitol, 0.075 M sucrose, 0.01 M KCl, 0.01 M Tris buffer, pH 7.4, 1.67 mM Na_2S and 1.4 mg protein/ml.

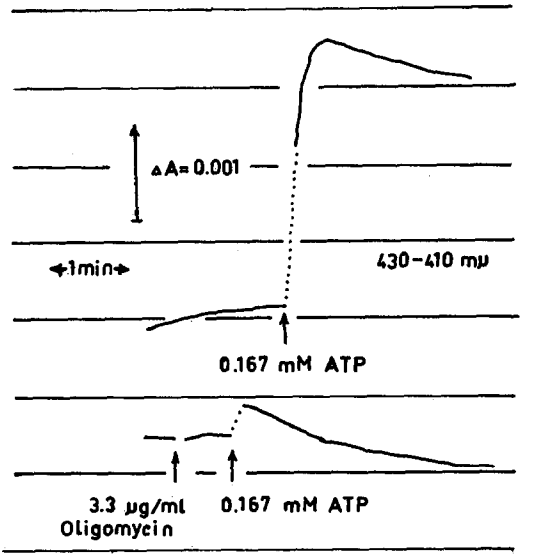
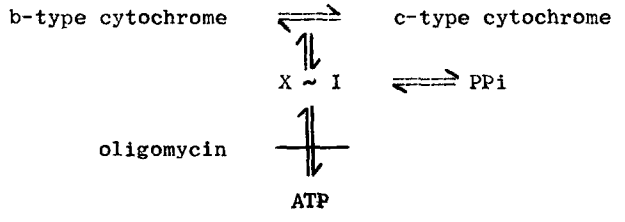


Fig. 4. Reduction of endogenous cytochrome b by ATP in rat liver mitochondria. Conditions as in Fig. 3.

insensitive reaction pathway in various mitochondrial respiratory systems indicates that PPi may be closely connected with intermediate stages of energy transfer also in oxidative phosphorylation. This may bring into focus the earlier demonstrations by Racker (1962) that a fraction containing a "coupling factor" F_3 and a pyrophosphatase activity may be prepared from mitochondria of higher animals (beef and rat) and by Heldt (1966) that in rat liver mitochondria appreciable amounts of ^{32}Pi are incorporated from inorganic orthophosphate into, among other compounds, inorganic pyrophosphate.

To summarize, it has been shown that PPi in its function as energy donor in photophosphorylating chromatophores allowed the first exact identification of a cross-over point (coupling site) in a photosynthetic electron transport chain, and that the ability of PPi to serve as a biological energy donor is not restricted to photosynthetic systems but extends also to respiratory electron transport systems of both lower and higher organisms. The following sequences of electron transport and energy transfer (minimum scheme):



thus appear to exist both in photophosphorylation and oxidative phosphorylation systems.

Full accounts of this work are in preparation.

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