Interaction of Photophosphorylation and Electron Transport Systems in Bacterial Chromatophores

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Chromatophore fractions isolated from photosynthetic bacteria can catalyze light-dependent phosphorylation of adenosine diphosphate to triphosphate and also a variety of light-activated electron transfer reactions, including photoreduction and photo-oxidation of dyes and pyridine nucleotides. Whether or not phosphorylation and such electron transport are independent, can occur simultaneously, or are "coupled" in some manner has been the subject of several investigations. Analyses of data from such studies have been complicated because the bacterial particles can carry out a vigorous light-dependent phosphorylation in the absence of any detectable oxidation-reduction change or substrate consumption ("cyclic" photophosphorylation); an obligate coupling of photochemical electron transport to phosphorylation has not been apparent. It is worth noting that the concept of coupling was developed from studies of mitochondrial phosphorylation in which a substrate is not only required but simultaneously oxidized; such a concept may not be germane to photophosphorylation systems supplied radiant energy. Furthermore, until the recent discovery of ferredoxin-mediated cyclic phosphorylation in chloroplasts, photophosphorylation systems of both plants and bacteria had been characterized by a general lack of sensitivity to classical uncoupling agents.

The present communication describes experiments resulting from the recent observation that bacterial chromatophores undergo induction periods in certain light-activated electron transfer reactions. During the induction period, the particles can photophosphorylate but cannot transfer electrons to added acceptors. Once induction is completed, the particles can photoreduce added electron acceptors, but stop phosphorylating. Hence, under certain conditions, the phosphorylation and electron transport reactions of chromatophores are not in any sense coupled but, on the contrary, appear to compete with each other for energy provided by the primary photochemical process.

EXPERIMENTAL PROCEDURE

The method of preparation of chromatophores from *Rhodo- spirillum rubrum* and assays for photophosphorylation and photoreduction have been previously described. Conventional spectrophotometric equipment was used in all experiments.

RESULTS

In studies on the photoreduction of the low potential electron acceptor system, methyl viologen-DTN, light energy was required when the oxidation-reduction potential of the electron donor couple was poised at a level insufficient to reduce the disulfide acceptor. Therefore, this reaction is an energy-requiring photoreduction. One can ask whether this energy interacts in any way with the photophosphorylation system. In Fig. 1, data show the effects on disulfide photoreduction of a potent inhibitor of photophosphorylation, antimycin A, with that of an activator of phosphorylation, phenazine methosulfate. The pattern of inhibition of the two processes shows an inverse relationship; the phosphorylation inhibitor stimulates photoreduction, whereas the phosphorylation activator inhibits the reaction. This pattern has been observed with a variety of compounds and conditions. The data in Fig. 1 also illustrate the typical induction period in disulfide photoreduction characteristic of chromatophores. It should be noted that these phosphorylation inhibitors and activators had little effect on the induction period. The effects of reagents of this type were observed primarily after the reaction had begun.

In contrast to inhibitors and activators of photophosphorylation, which exerted their effects principally on the ultimate rate of disulfide photoreduction, a variety of classical chemical uncoupling agents influenced the induction period. For example, data in Fig. 2 show that carbonyl cyanide methochlorophenol-hydrazone, pentachlorophenol, and gramicidin all shortened the induction period. At no concentration did the chemical uncoupling agents completely eliminate induction, which suggests that a period of electron flux is required for the uncoupling agent to act. On the other hand, media of high ionic strength completely eliminate induction (Fig. 2).

The time course of photophosphorylation and of disulfide photoreduction have been measured on identical preparations of chromatophores. As illustrated in Fig. 3, the two processes do not take place simultaneously; in fact, disulfide photoreduction begins at a time closely coinciding with cessation of phosphorylation activity. Under these conditions, the preparation could not maintain a high rate of photophosphorylation and could not initiate photoreduction until the preliminary induction period, characterized by a high phosphorylation rate, had been completed.

The abbreviations used are: DTN, 5,5'-dithiobis(2-nitrobenzoic acid); DCI, 2,6-dichlorophenolindophenol; TPD, tetra-methylphenylenediamine; O.D., optical density of enzyme preparation at 880 μm (the absorption maximum of bacteriochlorophyll bound to *R. rubrum* chromatophores).
completed. Thus, the particles appear to be chronically committed to photophosphorylation as a primary act.

Chromatophores in media of high ionic strength show no induction and cannot photophosphorylate acceptors but can photoreduce compounds, whereas particles in media of low ionic strength photophosphorylate and have induction periods in electron transfer (10). Spectra of preparations in these two conditions were compared. Fig. 4 shows a difference spectrum of chromatophores in media of high versus low ionic strength.

**Fig. 1.** Effect of photophosphorylation inhibitor and activator on disulfide photoreduction. For the antimycin experiment, 20 μg of antimycin was added to the reaction mixture containing 20 μmoles of ascorbate, 0.1 μmole of DCI, 2.0 μmoles of methyl viologen, 1.0 μmole of DTN, and 0.1 ml of chromatophores (O.D.880 45) in 3.0 ml of 0.2 M potassium phosphate, pH 7.5. For the phenazine methosulfate experiment, 0.05 μmole of phenazine methosulfate (PMS) was added to a reaction mixture containing 10 μmoles of ascorbate, 0.2 μmole of DCI, 0.5 μmole of methyl viologen, 1.0 μmole of DTN, and 0.1 ml of chromatophore preparation (O.D.880 44) in 3.0 ml of 0.2 M Tris buffer, pH 7.5. Reactions were done aerobically in white light of 1000 foot-candle intensity.

**Fig. 2.** Effect of chemical uncoupling agents on disulfide photoreduction. Reaction mixtures contained, in 3.0 ml of 0.1 M phosphate buffer (pH 7.5), 10 μmoles of ascorbate; 0.2 μmole of DCI; 0.5 μmole of methyl viologen; 1.0 μmole of DTN; and 0.1 ml of ionic strength of 0.6 M. Each cuvette contained 0.1 ml of particles (O.D.880 180) in 3.0 ml of potassium phosphate buffer, pH 7.5. The ionic strength of the blank cuvette was 0.6, and of the sample cuvette, 2.0. The spectrum contains peaks characteristic of R. rubrum cytochrome c, indicating that this hemoprotein is more reduced in media of high ionic strength, i.e. under nonphosphorylating conditions. In addition, peaks at about 300 and 900 μm appear. Correction of the spectrum for light scattering did not eliminate the maxima.

The light-activated transfer of electrons from ascorbate-DC1 and ascorbate-TPD to oxygen has also been studied in an effort to relate these electron transfer processes to photophosphorylation. Data in Fig. 5 compare these two reactions at high and low ionic strength. The ascorbate-DC1 reaction has an induction and ascorbate-TPD does not; therefore, the two electron donor systems interact differently with the bacterial particles.
Fig. 5. Comparison of photo-oxidation of ascorbate-DCI and TPD (abbreviated TMPD here) by chromatophores at different ionic strengths. Reactions were run with different molarity phosphate buffers, pH 7.0, and 0.05 ml of chromatophores (O.D.880 24) in 3.0 ml of buffers containing 1.0 μmole of reduced dye. Reactions were followed spectrophotometrically in open cuvettes, with 1000 foot-candles of white light (tungsten bulbs).

Fig. 6. Demonstration of induction period in disulfide photoreduction with ascorbate-TPD (abbreviated TMPD here) as electron donor. The reaction mixture contained, in 3.0 ml of 0.2 M phosphate (pH 7.5), 10 μmoles of ascorbate; 1.0 μmole of DTN; 0.5 μmole of methyl viologen; 1.0 μmole of TPD; and 0.1 ml of chromatophores (O.D.880 36).

However, data in Fig. 6 show that the ascorbate-TPD system has a characteristic induction period in electron transfer to the low-potential acceptor DTN, as observed with ascorbate-DCI in this photoreduction. These results suggested that the two electron donor systems differ in their ability to interact with the energy capture systems for phosphorylation.

Data in Fig. 7 show that the photophosphorylation system does respond differently to the presence of these two electron donor couples. Photophosphorylation could not be maintained operative in the presence of ascorbate-DCI, whereas ascorbate-TPD promoted accelerated phosphorylation for a longer time. Recently, oxidized DCI was shown to be an uncoupler of chloroplast photophosphorylation (12).

DISCUSSION

The present results suggest a means of resolving a number of conflicting reports that have appeared in the literature regarding coupling of electron transport to photophosphorylation in bacterial systems. In the initial study of photochemical pyridine nucleotide reduction by bacterial chromatophores, Frenkel (4) stated that phosphorylating conditions inhibited the process. Subsequently, Vernon and Ash (6) pointed out the apparent independence of the two processes, although they did not follow the time course of the reactions. Horio, Yamashita, and Nishikawa (13) showed that photoreduction of NAD was influenced by a variety of phosphorylation reagents. Nozaki, Tagawa, and Arnon (7) demonstrated a coupled noncyclic phosphorylation in chromatophores, but Bose and Geit (8) disputed this result. It now appears that some of the previously contradictory reports may have resulted from the additional complication that, under certain conditions, the process of electron transport can alter the relative activity of electron transport and photophosphorylation; in fact, a number of observations made in the present investigation suggest that uncoupling is a dynamic process. As illustrated in Fig. 3, if one sampled the reaction mixture after 20 to 30 minutes of illumination, as is commonly done, one could get a P to e ratio for two processes occurring at entirely different times. Thus, depending upon when the reaction mixture is sampled and upon such factors as ionic strength and the nature of the oxidation-reduction environment, it would be possible to conclude that one had “cyclic,” “coupled noncyclic,” or independent processes.

The studies reported here also suggest a means of evaluating various inhibitors of photophosphorylation as uncoupling agents versus inhibitors of electron transport. Inhibitors of photophosphorylation, like antimycin and Amytal, accelerate disulfide photoreduction but have little effect on the induction period.
In contrast is the effect of classical uncoupling agents which invariably shorten the induction period but, in general, have lesser or no effect on the photoinduction rate. Since all chemical uncoupling agents examined so far shorten induction, the induction period appears to be a useful assay of the extent of commitment to photophosphorylation. The "uncoupling time" in the sense of phosphorylation commitment is 6 to 8 minutes and is shortened 2 to 4 minutes by chemical uncouplers.

In studies on photophosphorylation in mitochondria coupled to electron transport mediated by high potential electron donors, Jacobs and Sanadi (14) and Jacobs (15) observed that photophosphorylation became uncoupled during electron transport from certain ion donors. Their work led to the extensive use of TPD as an uncharged electron donor capable of supporting phosphorylation in the oxygen terminal region of the aerobic respiratory system. The chromatophore photophosphorylation system is similar to the mammalian mitochondrion in its response to the same high potential electron donors. For example, ascorbate-TPD can maintain photophosphorylation and can donate electrons photochemically to oxygen without induction. In contrast, electron transport from ascorbate-DCI leads to suppression of photophosphorylation and this oxidation-reduction couple donates electrons to oxygen only after an induction period. Jacobs and Sanadi (14) developed the concept that special ionic factors maintained coupling in the oxygen terminal region of the respiratory chain and that transit of ionic electron donors disrupted coupling in this region. Present results with the photophosphorylation system in chromatophores are generally consistent with this concept. It seems especially significant, however, that whether or not the ascorbate-TPD couple interacts with the particle in a manner causing induction or immediate electron transfer depends upon the nature of the electron acceptor—either oxygen or DPN. This result suggests that, although both reactions are light dependent, either the coupling relationship or the direction of electron transfer differ in the two systems, or both. Furthermore, it suggests that electron transport mediated uncoupling may be "directional."

The data presented here support the view that photophosphorylation driven by bacteriochlorophyll, analogous to that driven by chlorophyll a in plant preparations (16), competes with added electron acceptors for the energy provided by the primary photochemical process. This competition appears to be the key to the coupling problem in bacterial photophosphorylation. The nature of this coupling is quite different from that of oxidative phosphorylation in which it is clear that energy is derived from an oxidation. In the bacterial chromatophore, chemical uncouplers tend to favor a condition in which energized substrates from adenosine triphosphate synthesis to photochemical electron transfer reactions, apparently by diversion of energized components formed by the primary photochemical process. This competition is regulated by ionic factors and the oxidation-reduction environment in a manner resembling "coupling."

The chromatophore particles, as usually isolated in media of low ionic strength, appear strongly "committed" to photophosphorylation but undergo, in the presence of ionic high potential electron donors, an electron transport-mediated process analogous to uncoupling. This process results in suppression of adenosine triphosphate formation and initiation of light-induced electron transfer reactions, apparently by diversion of energized components from adenosine triphosphate synthesis to photochemical reductions.

REFERENCES

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