Biochimica et Biophysica Acta, 387 (1975) 129–134

BBA 46889

ELECTRON TRANSPORT IN AN IN VITRO-RECONSTITUTED BACTERIAL PHOTOPHOSPHORYLATING SYSTEM

AUGUSTO F. GARCIA*, GERHART DREWS** and MARTIN D. KAMEN***

Institut für Biologie II der Universität Freiburg, Lehrstuhl für Mikrobiologie, D-78 Freiburg (G.F.R.)

(Received September 3rd, 1974)

SUMMARY

Photooxidation of endogenous cytochrome(s) *c*, photoreduction of endogenous cytochrome(s) *b* and photobleaching of bacteriochlorophyll have been demonstrated in an in vitro reconstituted system, previously demonstrated to support photophosphorylation. The kinetic responses of these redox reactions to substrate and antimycin A in these particles are characteristic of electron transport processes, and strongly support the contention that all, or a part of, the oxidative phosphorylation electron transport pathway can be coupled to reaction center photopigment complex in a manner which supports photophosphorylation. In addition, a succinate-supported light dependent reduction of NAD^+ was found.

INTRODUCTION

Recently, we have described a reconstituted in vitro photophosphorylation based on an uncoupled membrane fraction from a phototrophic-negative *Rhodopseudomonas capsulata* mutant ($A1a^-$) potentiated by a reaction center preparation from its phototrophic positive revertant ($A1a^+r$) together with coupling factor [1]. This system, which provides an opportunity to probe further into functional relationships between photoinduced electron transport and phosphorylation, affords an approach to clarification of the molecular basis for photophosphorylation coupled to the electron transport pathway. For recent proposals concerning the redox chain components participating in photophosphorylation of this species see ref. 2.

In this paper, we report qualitative evidence for light-induced physiologically significant redox reactions associated with our active reconstituted photophosphorylation complex. Previously evidence has been presented that in a similar system, derived

Abbreviations: LDAO, lauryldimethylamine oxide.

* Present address: Fundación Bariloche, Departamento de Biologia, San Carlos de Bariloche, Rio Negro, Argentinia.

** To whom reprint requests should be sent.

*** Present address: Department of Chemistry, University of Southern California, Los Angeles, Calif. 90024, U.S.A.

from *Rhodopseudomonas spheroides* membrane, bound cytochromes *b* and *c* participate in photoinduced cyclic electron transport activated by reaction center bacteriochlorophyll [3].

MATERIALS AND METHODS

The cells of *Rps. capsulata* were grown as described previously [1]. The reconstituted particles were prepared from (1) photosynthetic inactive, bacteriochlorophyll-free membranes of the mutant strain $A1a^-$, (2) reaction centers from membranes of the phototroph positive revertant $A1a^+$ *r* and, where indicated, of (3) coupling factor from the $A1a^-$ strain. In a standard mixture 70 mg of protein from $A1a^-$ membranes were mixed with 3–5 mg protein of coupling factor and 5 mg protein of reaction center. The membranes, reaction centers and coupling factors were prepared also as previously noted [1].

Light-induced changes were measured with a Perkin-Elmer double beam spectrophotometer, Model 356, fitted with an attachment for cross illumination and using a special interference filter with 98% transmission at the maximum 840 nm and a band width of 280 nm between 700 and 980 nm. Scattered light was minimized by means of a Schott BG 38+KG 3 (7 mm) light filter combination placed between the sample and the photomultiplier. The cytochrome *c* type reactions were measured at 551 with 540 nm as reference wavelength, cytochrome *b* type at 561/570 nm and reaction center bacteriochlorophyll at 598/575 nm [2].

The reaction mixture contained in a final volume of 3.0 ml: 8 μmol $MgCl_2$; 40 μmol glycylglycine; 0.5 ml of 87% glycerol and, when indicated, 5 μg/ml of antimycin A (Serva, Heidelberg), final pH 7.9. NAD^+ photoreduction: the reaction mixture contained in a final volume of 2.83 ml: 4 μmol $MgCl_2$; 36 μmol glycylglycine; 3 μmol NAD^+; 25 μmol sodium succinate; final pH 7.9. In some preparations the complete photophosphorylating system as described in [1] was used. Bacteriochlorophyll was determined according to Clayton [4].

RESULTS AND DISCUSSION

Light-induced redox changes of cytochromes

Fig. 1 shows the light-induced changes measured at three different wavelength pairs which distinguish cytochrome *c* type, cytochrome *b* type and the bacteriochlorophyll reaction center in the presence and in the absence of added succinate. It has been suggested [5] that succinate and other reducing agents may poise the electron transport system of the photosynthetic membrane at a redox potential optimum for coupled electron flow. As can be seen below, our observations with the reconstituted system are consistent with this suggestion. Our LDAO-treated reaction center preparations which should be low in cytochrome content [6] show marked rapid photooxidation of cytochrome(s) *c* and photoreduction of cytochrome(s) *b*. Hence, their content of cytochromes was still sufficient to allow observation of these redox absorption changes. As expected the reaction center preparations were not responsive to succinate and did not show any reactions either on the substrate or on the oxygen side of the respiratory chain.

In the reconstituted particles the redox-changes of photooxidized cytochrome(s) *c* and photoreduced cytochrome(s) *b* under steady-state conditions were found to be

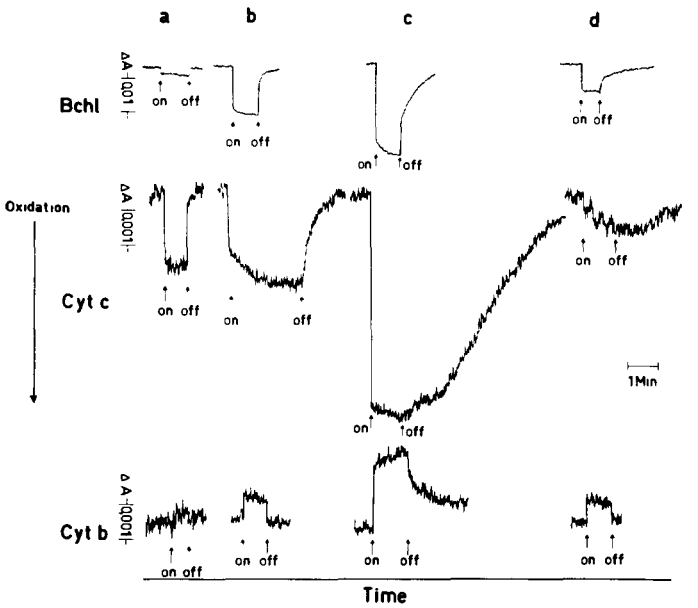

Fig. 1. Light-induced absorbance changes. (a) Phototrophic positive membrane A1a^+r; 37.2 μg bacteriochlorophyll; (b) reconstituted system, 2.32 μg bacteriochlorophyll; (c) reaction center preparation, 2.5 μg bacteriochlorophyll; (d) reconstituted system without succinate; 2.32 μg bacteriochlorophyll; a–c contained 15 μmol succinate; final volume, 3.0 ml.

smaller, but the kinetics of the light-off redox changes differed. As expected for more efficient electron transport consequent on reconstitution, rereduction of cytochrome(s) *c* and reoxidation of cytochrome(s) *b* in the dark were accelerated. Some quantitative differences in the responses of the three systems may be noted. The bleaching reaction appeared to increase in extent and heterogeneity in the order: A1a^+r, reconstituted system, reaction center. Redox changes of the cytochromes showed the same behaviour. One may interject the comment that the phototrophic-positive membrane is probably better organized for electron transport as well as coupled phosphorylation, so that it should respond with lesser redox changes in steady state, but also faster kinetics, both for light-on and light-off redox changes than would be expected for the reconstituted system. Similarly, the reconstituted system does better in this respect than the reaction center preparation in which disorganisation of the cytochrome complex relative to the photopigment complex as well as for access to substrate is greatest. It may be noted that succinate greatly enhances the cytochrome(s) *c* photooxidation in the reconstituted particles whereas cytochrome *b* photoreduction is unaffected. Evans and Crofts [2] have shown that succinate is more efficient in reducing the cytochrome *c* pool than the cytochrome *b* pool. The observations summarized in Fig. 1 confirm these results.

We interpret the change in the light-off kinetics as evidence of effective interaction between the reaction center and the phototrophic negative membrane correlated with coupling between the bacteriochlorophyll photooxidation and the cytochrome redox reactions. In the reaction center preparation it is clear there is no dependence between the two processes. Thus the kinetics of the cytochrome (s) *c* and *b* reactions in

the reconstituted system closely resemble those observed to occur in the phototrophic positive membrane.

Pigment photooxidation

Again some differences are readily apparent between the reaction center the recombined particles reactions. The magnitude of the changes in the latter decreases compared to those of the reaction center. During the light-on process, the reaction center shows a biphasic reaction which is not altered by the addition of succinate.

On the other hand the recombined particles do not show this biphasic light-on reaction. In the absence of succinate the light-off kinetics is biphasic. The addition of succinate in this latter case accelerates this process of rereduction.

We interpret this effect of succinate on the reconstituted system as evidence of the formation of a modified operative electron transport system consequent on recombination of reaction center and phototrophic negative membrane. Moreover in the reaction center preparation the rereduction most probably represents a charge recombination involving the reaction center bacteriochlorophyll and the pool of primary and secondary acceptors.

Effect of antimycin A

It is well known that antimycin A interferes with photosynthetic electron transport between cytochrome *c* and cytochrome *b* [2, 5]. We have compared the effect of antimycin A on the electron transport system of the phototrophic positive membrane and on the reconstituted particles. It should be noted that with the concentration used antimycin A showed no effect on the reaction center-mediated reactions. In reaction center preparations the electrons move mainly between reaction center

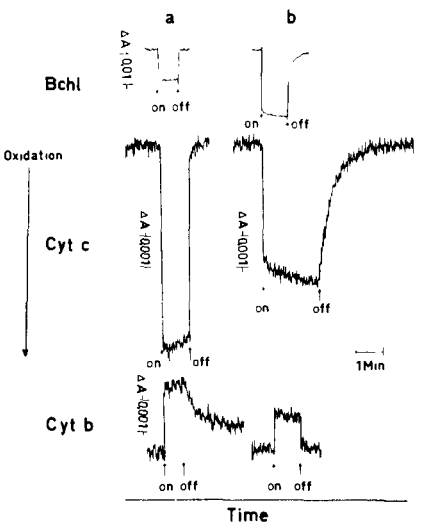

Fig. 2. Effect of antimycin A on light induced absorbance changes. (a) Phototrophic positive membrane A1a^+r; 37.2 μg bacteriochlorophyll per 3.0 ml; (b) reconstituted system; 2.32 μg bacteriochlorophyll per 3.0 ml. Antimycin concentration 5 μg/ml. The preparations were preincubated 30 min in the dark under N_2 atmosphere prior to illumination.

bacteriochlorophyll and primary acceptor. The cytochromes are accessible to electrons from bacteriochlorophyll, but are not tightly coupled. In consequence, no real cyclic electron flow is observable.

On the other hand, the effects were similar for both the phototrophic positive membrane and the reconstituted system. Thus, increases in the extent of all three reactions studied were evident (Fig. 2). In the phototrophic-positive membrane this may be interpreted as evidence of the existence of a cyclic electron pathway, most probably also present in the reconstituted particles as a result of recombination. A difference in the redox kinetics of cytochrome(s) *b* is the only exception to the overall observation of grossly similar effects produced by antimycin A, in that the phototrophic positive membrane shows heterogeneity in both light-on reduction and light-off reoxidation. Several explanations may be given based either on differences in cytochrome(s) *b* composition or accessibility, or alternatively on differences in pool sizes of reductants which interact with the cytochrome *b* pools.

Coupled system

As seen in Fig. 3 the reconstituted system in the presence of added coupling factor shows an acceleration of the light-off kinetics of the cytochrome *c* and pigment photooxidations. Also the extent of the reaction center bleaching is greater. The effects on cytochrome redox changes can be rationalized as indicating that addition of coupling factor shifts the steady state of photopigment and cytochrome(s) *c* to more reduced redox levels while affecting those of cytochrome(s) *b* in the opposite manner, again in harmony with the supposition that redox poising is an important factor in the coupled system.

NAD^+ photoreduction

We have found a succinate-supported light dependent reduction of NAD^+. The rate of the reduction was extremely low (5.9 and 16.8 nmol/h per mg bacteriochlorophyll as measured in two separate experiments). However, it is known that this reaction proceeds at a very slow rate in photoheterotrophically grown *Rps.*

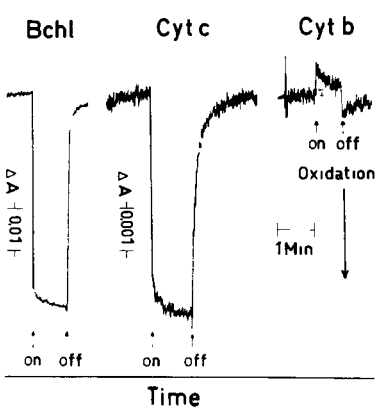

Fig. 3. Light-induced absorbance changes of the reconstituted system in the presence of coupling factor. The reaction mixture was the same as used for photophosphorylation; 4.24 μg bacteriochlorophyll per 3.0 ml final volume.

capsulata (1.5 μmol/h per mg bacteriochlorophyll [7]). As neither the reaction center itself nor the phototrophic negative membrane alone support this reaction, this is further evidence that the coupling between the phototrophic negative membrane and the reaction center bacteriochlorophyll can produce a physiologically significant reaction.

CONCLUSION

We have shown that there are marked effects on light-induced endogenous cytochrome states as well as in the photobleaching of bacteriochlorophyll when the reaction center is recombined with a phototrophic negative membrane.

The behaviour of this recombined system in the presence of succinate and the electron transport inhibitor antimycin A, clearly establish very similar behaviour of the reconstituted system and the phototrophic positive membrane.

The recombined particles also mimic well the native photosynthetic membrane in that they are able to perform a physiological reaction, such as NAD^+ photoreduction, although at a diminished rate.

Together with our previously described light induced ATP formation in this in vitro system, the present results encourage the hope that these reconstituted particles provide a cell-free electron transport coupled photophosphorylation complex, which will be proved useful for further clarification of the relationship between the oxidative and photosynthetic electron transport reactions.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Deutsche Forschungsgemeinschaft S.F.B. 46, to G.D. A.F.G. is a fellow of the Alexander von Humboldt Foundation. M.D.K. is a Senior U.S. Scientist Awardee of the Alexander von Humboldt Foundation. Partial support to M.D.K. by grants from the National Institutes of Health, U.S.A. (GM-18528) and National Science Foundation, U.S.A. (GB 360 19X) is recorded.

REFERENCES

1 Garcia, A. F., Drews, G. and Kamen, M. D. (1974) Proc. Natl. Acad. Sci. U.S. 71, 4213–4216
2 Evans, E. H. and Crofts, A. R. (1974) Biochim. Biophys. Acta 357, 78–102
3 Jones, O. T. G. and Plewis, K. M. (1974) Biochim. Biophys. Acta 357, 204–214
4 Clayton, R. K. (1963) in Bacterial Photosynthesis (Gest, H., San Pietro, A. and Vernon, L. P., eds), p. 498, Antioch Press, Yellow Springs, Ohio
5 Frenkel, A. W. (1959) Annu. Rev. Plant Physiol. 10, 53–70
6 Prince, R. C. and Crofts, A. R. (1973) FEBS Lett. 35, 213–216
7 Klemme, J. H. (1969) Z. Naturforsch. 24b, 67–76