Volume 216, number 2, 241-244 FEB 04723 June 1987

Does bacteriorhodopsin energize the membranes of animal mitochondria under light?

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Received 17 March 1987

A suspension of freeze-thawed mitochondria mixed with purple membranes from *Halobacteria* was illuminated with visible light. It was found that the light exposure prevented inhibition of succinate oxidation. The illumination also led to a decrease in inhibition of the rate of ferricyanide reduction by rat liver mitochondria in the presence of succinate. Both phenomena are explained by the fact that oxalacetate inhibition of succinate dehydrogenase is prevented by light-induced energization of mitochondrial membranes due to the contact with purple membranes.

Purple membrane; Mitochondria; Succinate dehydrogenase

1. INTRODUCTION

Purple membranes of *Halobacterium halobium* (PM), which contain bacteriorhodopsin (bR), may interact with the cell membranes of non-photophosphorylating microorganisms, thus supplying the latter with energy under light [1]. The mechanism of transformation of the energy of light into the energy of life is investigated. It is shown that the light energy is transformed into a electrochemical proton gradient on the planar phospholipid membrane [2] and liposomes [3] and subbacterial particles [4] containing bR. It may be suggested that in biological systems the transformation of light energy by bR upon its contact with natural membranes occurs via a mechanism similar to that in model systems.

Among biological membranes, those in mitochondria are of particular importance in this respect. It would be of interest to observe the energy accumulation in a mitochondrial membrane containing bR on exposure to light. No similar experiments have been previously described, probably because the self-potential of a mitochondrial membrane is much greater than the photo-dependent potential generated by bR on the membranes of intact mitochondria. Thus, it was necessary to use preparations of mitochondria with a low membrane potential, as well as employing a test reaction sensitive to the energy state of mitochondria. Such an attempt was made by us in the preliminary experiments [5]. Here, the oxidation of succinate in the respiratory chain was used as an energy-sensitive test response in mitochondria.

The activity of succinate dehydrogenase (SDH) is known to decrease in low-energized mitochondrial preparations due to inhibition of the enzyme by oxalacetate formed from succinate [6,7] and to the conformational change of the enzyme [8]. Freeze-thawed mitochondria (FTM) from rat liver and, especially, from pigeon heart may serve as a very low-energized preparation in which variations in rate of succinate oxidation measured by oxygen consumption are readily detectable. FTM are also convenient for access of PM to the inner membrane, facilitated due to disruption of the outer membrane of the particles.

The transfer of reducing equivalents to ferricyanide (FC) occurring with a higher rate than

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/87/$3.50

respiration is much more dependent on the energy state of mitochondria than consumption of oxygen [9]. The use of a more sensitive FC test enabled us to observe the influence of the photochemical process on succinate oxidation in a less damaged preparation than FTM, namely mitochondria aged in an ice-water bath over several hours. A suitable combination of the appropriate mitochondrial preparation and an energy-dependent test response provides registration of the bR-induced effect of light in this work. The accelerations in the rate of succinate oxidation are explained as a result of the increase in energy production in bR-treated mitochondria under light.

2. MATERIALS AND METHODS

Rat liver mitochondria were isolated as described in [10] with some modifications. Pigeon heart mitochondria were prepared by differential centrifugation at $g_1 = 330 \times g$ and $g_2 = 14000 \times g$ in a medium containing 0.225 M mannitol, 0.075 M sucrose, 1 mM EDTA, 10 mM Tris-HCl, 1% albumin, pH 7.5. FTM were obtained by double freeze-thawing at room temperature without further separation. The composition of the incubation media and other conditions are given in the figure legends.

PM were obtained according to [11] and possessed no succinate dehydrogenase activity. A water suspension of PM, pH 7.0 (10^{-3} M bR, corresponding to 5×10^{12} PM/ml), was added to the incubation medium just before the addition of mitochondria, so that the number of PM exceeded that of mitochondria 2–5-fold [12]. The adhesion of PM to mitochondria was tested by centrifuging the mixture of PM and mitochondria at $g = 14000$, sufficient for sedimentation of mitochondria but not for PM. No PM were observed in the supernatant spectrophotometrically. Electron-microscopic analysis confirmed the absence of free PM in the mixture of suspensions of mitochondria and PM.

Respiration was determined polarographically. FC reduction was registered potentiometrically with a pH electrode. Since oxalacetate-induced inhibition is known to be a temperature-dependent process, all experiments were performed in thermostatted cells ($t = 26°C$) illuminated through a water filter under temperature control. Temperature variations were less than 0.3°C and did not induce appreciable changes in the respiration rate of mitochondria.

Suspensions were illuminated by light from a projector (100 W) passed through a G-17 cut filter.

3. RESULTS AND DISCUSSION

In the presence of succinate the respiration rate of FTM from rat liver decreases, thus demonstrating SDH inhibition. This is typical of low-energized mitochondrial preparations. To make the decrease more pronounced, we used suspensions at concentrations lower than usual (1 mg mitochondrial protein/ml, corresponding to $\simeq 10^{10}$ mitochondria/ml [12]). This also enabled us to expose the samples for a much longer period until oxygen was completely exhausted (fig.1). Addition of glutamate to the FTM suspension prevented, to some extent ($\simeq 20\%$), the inhibition of FTM respiration, which indicates the participation of oxalacetate formed from succinate in the inhibition. Illumination of the suspension of rat liver FTM in the presence of PM resulted in 50% elimination of the inhibition of respiration as the initial respiration rate remained practically unchanged.

Pigeon heart mitochondria are known to be considerably less energized than those of rat liver, due

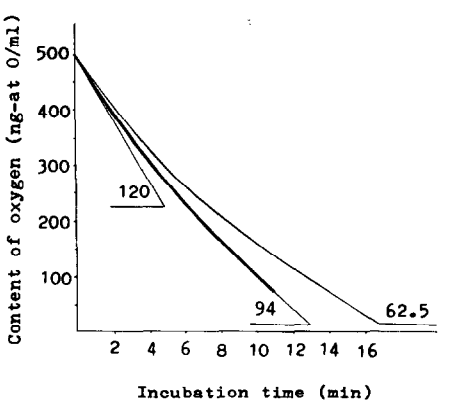

Fig.1. Oxygen consumption by freeze-thawed mitochondria from rat liver in the presence of purple membranes. The values of respiration rate in the dark (thin line) and under light (thick line) are indicated in ngatom O/min per mg protein. Incubation conditions: 0.15 M sucrose, 0.05 M KCl, 5 mM succinate, 5 mM Tris-HCl, pH 7.5. Protein concentration in a sample: 1 mg/ml; $t = 26°C$.

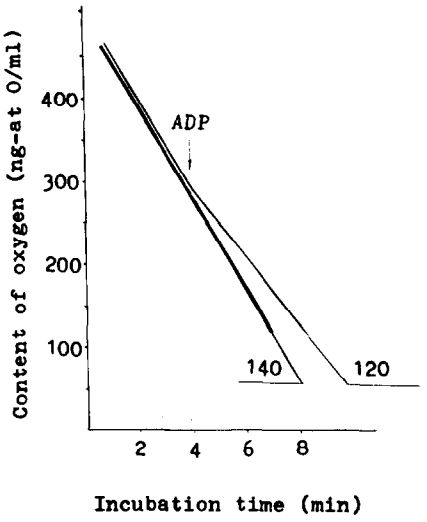

Fig.2. Oxygen consumption by freeze-thawed mitochondria from pigeon heart in the presence of purple membranes. 150 μM ADP added in the dark (thin line) and under light exposure (thick line). Values of respiration rate are indicated in ngatom O/min per mg protein. Incubation conditions: 0.225 M mannitol, 0.075 M sucrose, 1 mM EDTA, 3 mM KH_2PO_4, 5 mM succinate, 10 mM Tris-HCl, pH 7.5. Protein concentration in a sample: 0.7 mg/ml; $t = 26°C$.

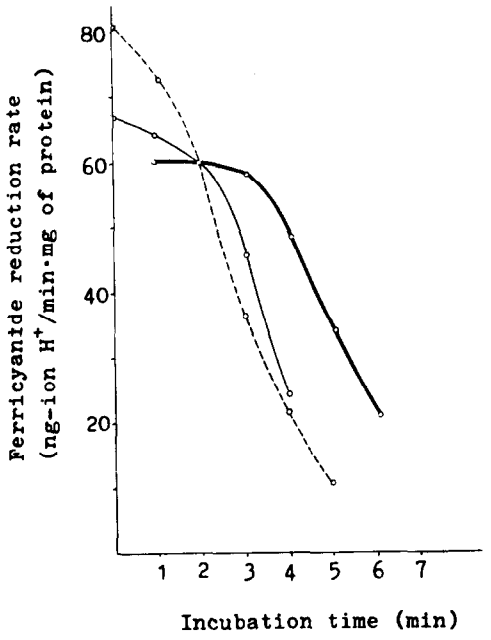

Fig.3. Time dependence of the ferricyanide reduction rate by aged mitochondria. Purple membranes absent (dashed line) and present in the dark (thin line) and under light exposure (thick line). Incubation conditions: 0.15 M sucrose, 0.05 M KCl, 5 mM succinate, 5 mM Tris-HCl, pH 7.5. Concentration of protein in a sample: 1.1 mg/ml; $t = 27°C$.

to the low level of endogenous substrates. Correspondingly, in pigeon heart FTM the inhibition of succinate was greater than in rat liver FTM. In particular, it was observed that, in the presence of succinate, ADP addition induces not stimulation but a decrease in respiration in pigeon heart FTM. Under light exposure of pigeon heart FTM with PM the inhibitory effect of ADP is completely abolished (fig.2).

The possibility that the light effect is not mediated by PM was excluded by experiments with FTM illumination without PM. In this case elimination of respiration inhibition was not observed; in contrast, a small additional (about 10%) decrease in respiration was registered. The latter may be due to membrane damage caused by light-induced activation of lipid peroxidation.

The possibility that the elimination of oxalacetate inhibition is due to heating during illumination was excluded by thermostatting of the cuvette (see section 2).

The experimental results described are manifested to different degrees in about half of the preparations. The distribution of the amplitudes of the effect and its absence are connected with variations in damage to the FTM. In some cases, in order to obtain the effect, additional freeze-thawing was necessary. In some cases the light effect may be abolished by additional freeze-thawing.

The light-induced PM mediated elimination of inhibition of succinate oxidation was reproduced in all experiments with aged rat liver mitochondria, which are less energized than intact mitochondria and more energized than FTM. In this case, the rate of FC reduction was registered by a pH electrode in mitochondria aged in an ice-water bath for 3–5 h. Fig. 3 (dashed line) shows that, without PM and illumination, FC reduction is strongly inhibited within several minutes. The other two curves show FC reduction by mitochondria in the presence of PM under illumination (thick line) and

without illumination (thin line). It may be pointed out that illumination prevents the initial decrease in FC reduction and provides stabilization of the level of reduction rate over several minutes before its final inhibition. In the dark this stabilization is not observed and inhibition occurs throughout the course of incubation. The difference between the light and dark rates is observed in each experiment, but its value varies. In the case shown, it reaches 100%. The lack of inhibition of FC reduction is a result of the elimination of inhibition of SDH under these conditions.

The described experiments demonstrated elimination of inhibition of succinate oxidation in several types of mitochondrial preparations under illumination in the presence of PM. Elimination of inhibition of succinate oxidation is known to be the result of energization of mitochondria. It may be due to pyridinenucleotide reduction, which is followed by conversion of oxalacetate to malate and, consequently, to the elimination of oxalacetate inhibition. Moreover, the reduction of the respiratory chain in the CoQ region induces a conformational change in SDH, decreasing its affinity for oxalacetate [13]. The decrease in initial rate of FC reduction under light in fig.3 may also be caused by enhanced coupling in the controlled state of respiration due to additional energization of mitochondria, induced by light in the presence of PM.

The light-induced energization of the mitochondrial preparation in the presence of PM may be explained by certain interaction of PM with mitochondrial membranes. Therefore, PM participate in the formation of the electrochemical proton gradient on the mitochondrial membrane in the region of its contact with PM. The occurrence of such an interaction suggests that the mechanism of transformation of light energy via bR is universal for both model and natural systems.

The results obtained demonstrate that the approach described can be successfully used for detection of the interaction between PM of *Halobacteria* and membranes of mitochondria.

ACKNOWLEDGEMENTS

The authors thank Professor M. Kondrashova for extensive discussions of the problem described in this paper and for help in formulating this manuscript.

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