Postillumination Adenosine Triphosphate Synthesis in Rhodospirillum rubrum Chromatophores

I. CONDITIONS FOR MAXIMAL YIELDS

(Received for publication, March 28, 1974)

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SUMMARY

The very low level of postillumination ATP synthesis in chromatophores was markedly stimulated when permeant anions (thiocyanate or perchlorate) or permeant cations (potassium in the presence of valinomycin) were added to the light stage. Although these compounds stimulated also light-induced proton uptake in chromatophores the pH dependence of both photoreactions was different. Proton uptake peaked at pH 6.5 while the amount of postillumination ATP was maximal when the light stage was carried out around pH 7.7. The increased yield of ATP at the more alkaline pH could not be explained by a slower decay of the high energy state at this pH, since the decay rate was faster at pH 7.7 than at pH 6.5.

The proton concentration gradient which is maintained across the chromatophore membrane in the light was also found to increase when the external pH was raised from 6.0 to 8.0. Only a minimal amount of postillumination ATP was formed when this gradient was below 2.1 pH units, but above this value the ATP yield rose steeply as a function of the increasing pH gradient. In light of these results it is suggested that in order to obtain a high yield of postillumination ATP synthesis in chromatophores two conditions are required: the particles have to be loaded with a sufficient number of protons and a light-induced pH gradient above a certain threshold value has to be maintained across their membrane. The low yield of postillumination ATP in chromatophores and the increase obtained by adding permeating ions, is thus explained by similar variations in the extent of the pH gradient, which exceeded the threshold value only in the presence of the permeating ions.

Illumination of chloroplasts in the absence of ADP and Pi resulted in accumulation of a high energy state or intermediate (Xe) which could be utilized for ATP synthesis in a subsequent dark reaction (1). The light stage of this two-stage or postillumination ATP synthesis in chloroplasts was found to have the same pH optimum as the light-induced H+ uptake (2–4). Also, addition of compounds which can serve as internal buffers markedly increased the extent of H+ uptake as well as the amount of postillumination ATP formation in chloroplasts (5, 6). It has, therefore, been proposed that the energy storage in the light stage of two-stage ATP synthesis resides in the protons concentrated inside the chloroplasts. This view was substantiated by the finding that ATP formation could be driven in total darkness by an artifically produced pH gradient (7).

Although light-induced H+ uptake was observed also in chromatophores, tests for postillumination ATP synthesis in these particles gave negative results (9, 10). Another marked difference between chloroplasts and chromatophores was recorded in the effect of various inhibitors on H+ uptake and photophosphorylation. Thus, addition of nigericin + KCl or NH4Cl resulted in inhibition of H+ uptake but not of photophosphorylation. These observations have been explained by the suggestion (13, 14) that chromatophores, unlike chloroplasts, are impermeable to Cl−. Therefore, light-induced H+ uptake results in chromatophores in the formation of an electrochemical proton gradient (15) which is composed of a much larger membrane potential component than in chloroplasts and a smaller pH gradient.

According to Mitchell (16) the membrane potential should be neutralized in the presence of permeant anions. Addition of SCN− and ClO4−, which were found to be permeant anions in Rhodospirillum rubrum chromatophores (17), has indeed led to a stimulation of light-induced H+ uptake (18). It was therefore of interest to see if this larger amount of proton storage might be used in enabling chromatophores to carry out postillumination ATP synthesis.

In this communication we demonstrate that postillumination ATP synthesis occurs in chromatophores in the presence of permeant anions as well as in the presence of valinomycin + KCl (19) which were also shown to stimulate proton uptake in these particles (13, 20). The amount of ATP synthesized in this system was similar to that reported in chloroplasts, but the optimal conditions required were found to be different, including a marked difference in the pH optimum of the light stage. These results indicate that the number of protons concentrated inside the chromatophores cannot be the only factor responsible for postillumination ATP synthesis in these particles.
Table I

Effect of various additions to light stage on postillumination ATP synthesis

The reaction mixture in the light stage contained in a total volume of 1.5 ml: 1.32 mM Tricine-NaOH, pH 7.7; 5.3 mM MgCl₂; 66 μM phenazine methosulfate; 330 μM succinate; 0.5 mM KCN; 180 μg of bacteriochlorophyll. The reaction mixture was illuminated at 25° for 45 s before injection into the dark reaction mixture which contained in a total volume of 0.75 ml: 120 mM Tricine-NaOH, pH 8.0; 1.06 mM ADP; 2.13 mM sodium phosphate (containing about 2 × 10⁶ cpm of [³²P]; 20 mM glucose; and 10 units of hexokinase. The dark reaction was stopped after 30 s by adding 0.26 ml of 30% perchloric acid. Appropriate dark controls have been subtracted from all values reported (see text).

<table>
<thead>
<tr>
<th>Additions to light stage</th>
<th>ATP formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2.3</td>
</tr>
<tr>
<td>NaSCN, 5 mM</td>
<td>9.0</td>
</tr>
<tr>
<td>NaSCN, 20 mM</td>
<td>11.6</td>
</tr>
<tr>
<td>NaClO₄, 5 mM</td>
<td>10.0</td>
</tr>
<tr>
<td>NaClO₄, 20 mM</td>
<td>14.0</td>
</tr>
<tr>
<td>NH₄Cl, 20 mM</td>
<td>1.2</td>
</tr>
<tr>
<td>KCl, 50 mM</td>
<td>2.6</td>
</tr>
<tr>
<td>KCl + valinomycin, 1 μM</td>
<td>42.5</td>
</tr>
</tbody>
</table>

Experimental Procedure

The growth of R. rubrum cells and the isolation and storage of chromatophores were as previously described (21). Bacteriochlorophyll was determined using the absorbance coefficient measured as outlined by Gromet-Elhanan (25).

Light-induced changes in the fluorescence of atebrin were assayed with a Metrom combined microglass electrode (type x) and a Radiometer pHM 22 pH meter and the signals were recorded on a Photovolt model 43 recorder. The examples were illuminated by a 500-watt filter. Confirming earlier observations (9, 10) very small amounts of ATP formed in nonilluminated controls as compared to less than 1 nmol per mg of bacteriochlorophyll which we have obtained in nonilluminated controls as compared to less than 1 μm valinomycin and 50 mM KCl were added to the light reaction mixture. In the columns designated “Dark” both stages were carried out in complete darkness. The numbers in the columns designated “Light” were obtained in experiment carried out as described in Table I.

Results

Confirming earlier observations (9, 10) very small amounts of postillumination ATP synthesis were observed in chromatophores when the light stage contained only an electron carrier and MgCl₂ (Table I). A 5 to 6-fold increase was observed when permeant anions, e.g. SCN⁻ or ClO₄⁻, were added to the light stage and a 20-fold increase was obtained with valinomycin + KCl. KCl alone had no effect and NH₄Cl, which under continuous illumination inhibited H⁺ uptake but not ATP synthesis (12), decreased the very low amount of ATP synthesized (Table I).

In order to determine the optimal conditions for this postillumination ATP synthesis in chromatophores several complicating reactions, which have been observed in chromatophores but not in chloroplasts, had to be eliminated. Thus, an active dark ATPase has been reported in chromatophores (26), and the kinetics of synthesis of ATP in the dark stage showed a pronounced decrease after 15 s (Fig. 1) which could be due to this activity. Addition of glucose and hexokinase eliminated this decrease and they were therefore routinely added to the dark reaction mixture.

Another complicating reaction is a dark ADP-P₁ exchange catalyzed by polynucleotide phosphorylase which has recently been reported in chromatophores (28). By the method employed here to measure ATP formation (23) [³²P]ADP formed via this exchange reaction in the presence of [³²P]P₁ will be recorded as ATP. This could account for the rather high amounts of 3 to 5 nmol of [³²P]esterified per mg of bacteriochlorophyll which we have obtained in nonilluminated controls as compared to less than 1 nmol per mg of chlorophyll observed in chloroplasts (24). To eliminate this complication all samples were heated in 1 N H₂SO₄ at 100° for 10 min (27). As can be seen in Table II this treatment reduced the dark control values to the level observed in chloroplasts. ATP formation would not be reduced by this treatment since all the ATP was trapped in the form of glucose 6-phosphate. If, however, inorganic pyrophosphate, which has been reported to be formed in R. rubrum chromatophores under continuous illumination (28), can be formed also in the two-stage system, it would have been hydrolyzed by heating in acid. The 3 nmol decrease observed after acid treatment in the last column...
of Table II could be due to such hydrolysis. This possibility was verified in the experiments illustrated in Table III. When no ADP was added to the dark reaction mixture and the acid treatment was eliminated 3.5 nmol of $^{32}$P, were esterified per mg of bacteriochlorophyll. Since this postillumination pyrophosphate formation accounted for less than 10% of the $^{32}$P, esterified in ATP all further experiments were run in the presence of ADP, glucose, and hexokinase and ATP synthesis was followed after acid treatment of all samples.

The temperature and pH optima of postillumination ATP synthesis in chromatophores were found to be different from those reported in chloroplasts. Much more ATP was formed in glucose, and hexokinase and ATP synthesis was followed after addition of ADP, hence effective (5). When such compounds were added to the light stage they were found to stimulate postillumination ATP formation around 6.3 which was also the optimum pH for ATP at this pH was very low, since the optimum pH for Xe formation remained around 8.0 where phenylendiamine was almost ineffective (Table IV). These results indicate that although suitable buffers can stimulate the yield of ATP in chromatophores as well as in chloroplasts, their presence does not change the observed pH optimum of Xe formation. In chloroplasts this was around 6.3 which was also the optimum pH for ATP formation with system tested.

<table>
<thead>
<tr>
<th>Additions to dark stage</th>
<th>$^{32}$P esterified with system tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dark</td>
</tr>
<tr>
<td>ADP, $^{32}$P</td>
<td>1.5</td>
</tr>
<tr>
<td>$^{32}$P</td>
<td>0.9</td>
</tr>
</tbody>
</table>

**TABLE III**

**Effect of addition of ADP to dark stage on amount of $^{32}$P esterified in two-stage phosphorylation**

Reaction mixtures were as in Table I in the presence of valinomycin and KCl, except that ADP was added only where indicated. Experiments were carried out as described in Table II. Samples from experiments performed in the presence of ADP were treated in 1 n H$_2$SO$_4$ for 10 min at 100° before being assayed. Experiments performed in the absence of ADP were not subjected to this acid treatment.

**TABLE IV**

**pH-dependence of postillumination ATP synthesis in presence of added buffers**

Reaction mixtures were as described in Fig. 2 in the presence of 20 mM NaSCN. Where indicated 1 mM p-phenylenediamine or 3 mM L-leucinamide were added to the light stage. The numbers in parentheses give the percentage of stimulation by the added buffer.
increasing with pH and must be large enough at pH 7.7 to counteract the unfavorable decay rate. It has recently been reported (29) that the yield of postillumination ATP formed in chloroplasts was very low when the pH gradient maintained across their membrane was below a certain threshold value. Above this threshold value the ATP yield increased as a function of the increasing pH gradient (ΔpH). Fig. 5 illustrates that the pH gradient across the chromatophore membranes rose as the external pH was varied from 6.0 to 8.0. The pH gradient was measured in this case by the quenching of atebrin fluorescence (30). A similar increase of the ΔpH with external pH was recently observed also by following the quenching of 9-aminoacridine.3 This increase was much steeper in the presence of SCN− so that in this case the ΔpH obtained at pH 7.0 was already higher than that observed in the control at pH 8.0 (Fig. 5). The relationship between the amount of postillumination ATP formed in chromatophores and the light-induced ΔpH is illustrated in Fig. 6.

Fig. 3. Kinetics of decay of Xe as a function of the pH in the presence of valinomycin and KCl. The reaction mixtures were as in Fig. 2 in the presence of 1 μM valinomycin and 50 mM KCl. After 45-s illumination the light was turned off for the indicated time interval before injection into the dark reaction mixture. Bchl, bacteriochlorophyll.

Fig. 4. Kinetics of decay of Xe as a function of the pH in the presence of SCN−. Reaction mixtures and conditions were as described in Fig. 3, except that 20 mM NaSCN were added to the light stage.

Fig. 5. Dependence of ΔpH on the external pH. The ΔpH was measured by following the light-induced quenching in the fluorescence of atebrin (25). The reaction mixture contained in a total volume of 3 ml: 3.3 mM MgCl₂, 33 μM phenazine methosulfate, 160 μM succinate, and 3.5 μg of bacteriochlorophyll. The pH was fixed with 30 mM of either Tricine-NaOH or Tricine-maleate. Where indicated 20 mM NaSCN were present. The ΔpH was calculated as the difference between the chromatophore internal pH and the external pH (30). The internal volume used in these calculations, 60 μl of H₂O per mg of bacteriochlorophyll, was determined by Schuldiner et al.4

Fig. 6. Postillumination ATP synthesis as a function of the light-induced ΔpH. The reaction mixtures were as described in Fig. 2 and the pH of the light stage was varied between 6.0 and 8.0. ΔpH values were taken from Fig. 5. Each point in the curve relates the ATP yield obtained at a specific pH in the light to the ΔpH recorded at the same external pH in the light in the absence (○) or presence (□) of 20 mM NaSCN. Fig. 6. As long as the ΔpH was below 2.1 a very low yield of ATP was obtained, but above this value the ATP yield rose sharply with the increasing ΔpH. It is therefore suggested that in chromatophores as well as in chloroplasts a highly active postillumination ATP synthesis can be demonstrated only when the pH gradient rises above a threshold value. But while in chloroplasts, a ΔpH above the threshold value is already obtained when the light stage is carried out at an external pH of 6.5 (29), the required threshold ΔpH in control chromatophores cannot be

3 M. Leiser and Z. Gromet-Elhanan, unpublished observations.

reached even at pH 8.0 and is surpassed in the presence of SCN− only when the pH of the light stage is above 7.0 (Fig. 5).

**DISCUSSION**

It has been reported by McCarty (19) that addition of valinomycin + KCl in the light stimulated both H+ uptake and postillumination ATP synthesis in subchloroplast particles. In the presence of these compounds, some postillumination ATP synthesis was observed also in chromatophores but the yield was much lower than in chloroplasts (19). The data summarized above demonstrate that such ATP synthesis does occur in chromatophores when either permeant anions or valinomycin + KCl are present in the light. Furthermore, under optimal conditions ATP yields approaching those observed in chloroplasts could be much lower than in chloroplasts (19). The data summarized presence of these compounds, some postillumination ATP synthesis reached even at pH 8.0 and is surpassed in the presence of SCN− only when the pH of the light stage is above 7.0 (Fig. 5).

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The dependence of postillumination ATP synthesis on a ΔpH above a certain threshold value as well as on a sufficient concentration of protons has been demonstrated by Schuldiner et al. also in chloroplasts (20). In chromatophores the ΔpH was calculated as the difference between their internal pH and the external pH in the light stage (Fig. 5). Schuldiner et al. included in their ΔpH calculations also the difference in pH between the light and dark stages (see Fig. 2 in Reference 20), but in chromatophores this pH difference did not seem to contribute to the ATP yield. For instance, at pH 6.0 in the light the large difference between this and the pH of the dark stage failed to improve the low yields of ATP, which seem to follow closely the ΔpH formed in the light stage only (Fig. 6). Indeed in chloroplasts a pH difference imposed in total darkness was found to initiate the so called acid-base phosphorylation (7), while in chromatophores a similar acid-base transition was inactive in driving ATP synthesis unless an additional source of energy was added (32).

Although the amount of postillumination ATP formed at pH 8.0 in the light stage was much larger in the presence of 20 mM NaSCN than in its absence (Table I and Fig. 2), no such difference was observed under continuous illumination (17, 18). In the presence of NaSCN the pH gradient was found to increase at the expense of the membrane potential (18), while the over-all proton motive force remained practically unchanged.* It was therefore suggested that under continuous illumination either the membrane potential or the pH gradient can drive ATP synthesis. In the postillumination set up, on the other hand, the membrane potential developed by H+ uptake seems to be much less effective than the pH gradient, since a large yield of ATP was obtained only when the pH gradient was increased at the expense of the membrane potential. The inactivity of this H+ driven membrane potential may be due to its fast decay (19) so that it is largely disipated by the time the chromatophores come in contact with the phosphorylation reagents in the dark stage.

Unlike the membrane potential formed solely by H+ uptake, a K+ diffusion potential formed in the dark has been proposed to stimulate postillumination ATP synthesis (19). It has indeed been recently reported that addition of valinomycin + KCl to the dark stage, resulted in an increase in the amount of postillumination ATP formed under suboptimal conditions in chloroplasts (29, 33), as well as in chromatophores (34). The large stimulation of postillumination ATP synthesis in chromatophores when valinomycin + KCl were added to the light stage (Table I and Fig. 2) seems therefore to be due to a dual effect: stimulation of H+ in the light and formation of a K+ diffusion potential in the dark. This double effect is, however, complicated since, as illustrated in the accompanying paper (34) the effect of the K+ diffusion potential has a different pH dependence than that of the light induced pH gradient.

**REFERENCES**

Postillumination adenosine triphosphate synthesis in Rhodospirillum rubrum chromatophores. I. Conditions for maximal yields.

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