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**On Light-Induced Phosphorylation and Oxidation of Reduced Diphosphopyridine Nucleotide in *Rhodospirillum rubrum***

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The highest values yet reported for the specific activity of bacterial light-induced phosphorylation, have been obtained with isolated chromatophores from the photosynthetic bacterium *Rhodospirillum rubrum*, which has been grown with alumina (Alcoa A-301) in order to rupture the intact cells. During the last year we have also used sand (Baker, purified) as an abrasive instead of alumina, and these preparations have usually shown a higher photophosphorylative activity when compared to alumina-ground preparations from the same batch of bacteria. However, the yield has been considerably lower. The reduced diphosphopyridine nucleotide oxidase (DPNH-oxidase) activity in "sand-preparations" and "alumina-preparations" has also been investigated. Its distribution between various fractions depends on the method of preparation. The DPNH-oxidase has been partly purified.


**Table 1.** Comparison of light-induced phosphorylation in preparations made with sand and with alumina. The values are given as µmoles orthophosphate esterified per hour per mg chlorophyll. Phosphorylation was determined as in Ref. The 25 000 g fraction was used.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Alumina</th>
<th>Sand</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>320</td>
<td>480</td>
</tr>
<tr>
<td>2</td>
<td>280</td>
<td>530</td>
</tr>
<tr>
<td>3</td>
<td>420</td>
<td>540</td>
</tr>
<tr>
<td>4</td>
<td>620</td>
<td>490</td>
</tr>
<tr>
<td>5</td>
<td>230</td>
<td>530</td>
</tr>
</tbody>
</table>

The cells were grown, harvested and ground with alumina according to previously described methods. When sand grinding was employed the procedure was as follows: the paste of wet bacteria was weighed and transferred to an ice-cold mortar. Cold sand in an amount equivalent to twice the weight of the bacteria was added, the mixture was ground for 3 min and subsequently extracted with ice-cold 0.2 M glycyglycine buffer, pH 7.4, 2 ml for each gram of bacteria. The extracted material was centrifuged 10 min at 10 000 g, the sediment discarded and the supernatant centrifuged 1 h at 25 000 g or 40 000 g. The latter force brings down all or almost all the colored material in contrast to its effect on "alumina preparations" where more than one third of the colored material normally remains in the supernatant. This indicates that "sand preparations" contain larger particles, presumably more "intact" chromatophores.

**Table 2.** Typical distributions of DPNH-oxidase activity in preparations from two batches of *Rhodospirillum rubrum*. The activity was measured at 340 mp in a Beckman DK2 spectrophotometer. The values are given as µM (µmoles per liter) DPNH oxidized per min per mg protein. The initial concentration of DPNH was 10⁻⁴ M.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Abrasive</th>
<th>Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25 000 g</td>
<td>100 000 g</td>
</tr>
<tr>
<td>5</td>
<td>Alumina</td>
<td>2.6</td>
</tr>
<tr>
<td>5</td>
<td>Sand</td>
<td>5.8</td>
</tr>
<tr>
<td>8</td>
<td>Alumina</td>
<td>10.0</td>
</tr>
<tr>
<td>8</td>
<td>Sand</td>
<td>21.0</td>
</tr>
</tbody>
</table>
Fig. 1. The stimulation by menadione on DPNH-oxidase activity in the supernatant after centrifugation at 100 000 g (○) and the
"100 000 g-fraction" (●). The DPNH-oxidation
was measured at 340 μM in a Beckman DK2
spectrophotometer. The reaction volume was
3 ml. The initial concentration of DPNH was
10⁻⁴ M. Sand preparation.

Table 1 gives a comparison of light-
induced phosphorylation in five prepara-
tions made with sand and alumina,
respectively, from five consecutive batches of
bacteria. As a complement to this table
it may be mentioned that in the former
kind of preparation an activity as high as
800 μmoles of orthophosphate esterified
per hour per mg chlorophyll (3 ml, 30°C) has
been obtained, in the latter the highest
value has been 650.

A marked difference between the two
types of preparations is found in the relative
distribution of the DPNH-oxidase activity in different fractions (Table 2). In "alumina preparations" it is high in the supernatant after centrifugation at 100 000

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