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1. Andersson, S., Collén, B., Kuylenstierna, U. and Magnéli, A. *Acta Chem. Scand.* **11** (1957) 1641.
2. Westman, S. and Magnéli, A. *Acta Chem. Scand.* **11** (1957) 1587.
3. Holmberg, B. and Dagerhamn, T. *To be published in Acta Chem. Scand.*

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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 ground with alumina (Alcoa A-301) in order to rupture the intact cells [1]. 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 photophosphorylating 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.[2] The 25 000 *g* fraction was used.

Batch	Alumina	Sand
1	320	480
2	280	530
3	420	540
4	620	490
5	230	530

The cells were grown, harvested and ground with alumina according to previously described methods [2]. 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 glycylglycine 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 mμ 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.

Batch	Abrasive	Fraction		
		25 000 *g*	100 000 *g*	Supernatant
5	Alumina	2.6	5.5	7.3
5	Sand	5.8	13.3	1.8
8	Alumina	10.0	4.3	11.1
8	Sand	21.0	3.3	2.2

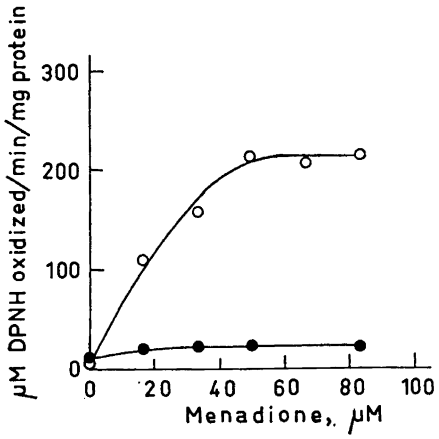

Fig. 1. The stimulation by menadione on DPNH-oxidase activity in the supernatant after centrifugation at 100 000 *g* (O) and the "100 000 *g*-fraction" (●). The DPNH-oxidation was measured at 340 mμ in a Beckman DK2 spectrophotometer. The reaction volume was 3 ml. The initial concentration of DPNH was 10^{-4} M. Sand preparation.

Table 1 gives a comparison of light-induced phosphorylation in five preparations 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 *g*, but in "sand preparations" the activity in the supernatant is quite low, most of it being found in the chromatophores (washed or unwashed). This is taken as a second indication that the sand-method causes less disruption of chromatophore structure than the alumina-method.

The DPNH-oxidase is stimulated by menadione, as is shown in Fig. 1. It is seen that the stimulation is much higher in the supernatant than in the chromatophore fraction. Possibly the particle-bound DPNH-oxidase is not as accessible to menadione as the solubilized activity in the supernatant.

The DPNH-oxidase in the supernatant after centrifugation at 100 000 *g* has been partially purified by ammonium sulfate fractionation. The fraction between 35 and 45 % saturation contained the highest activity per unit protein and gave, when dialyzed against distilled water, through which a continuous, oxidizing air-stream was led, a precipitate. This could be separated by centrifugation and dissolved in either 0.2 M glycylglycine buffer of pH 7.4 or by adding a small volume of DPNH in substrate amounts. The product so obtained gave a 4-fold increase in specific DPNH-oxidase activity as compared to the supernatant. Horio and Kamen [3] have recently reported DPNH-oxidizing activities with a citrate extract from whole lyophilized bacteria. Their most active fraction came from 45-65 % saturation with ammonium sulfate. They got a 10-fold purification of the DPNH-2,6-dichlorophenol-indophenol reductase activity, however, with complete loss of DPNH-oxidase activity.

Further studies are in progress and a more detailed account will be published.

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1. Smith, L. and Baltscheffsky, M. *J. Biol Chem.* **234** (1959) 1575.
2. Baltscheffsky, H. *Biochim. et Biophys. Acta* **40** (1960) 1.
3. Horio, T. and Kamen, M. *Biochim. et Biophys. Acta* **43** (1960) 382.

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