SHORT COMMUNICATIONS

BBA 43162

p-Phenylenediamines as electron donors for photosynthetic pyridine nucleotide reduction in chromatophores from *Rhodospirillum rubrum*

Photosynthetic pyridine nucleotide reduction by chromatophores from photosynthetic bacteria at the expense of different electron-donor systems has been demonstrated in a number of laboratories[1–5]. We wish to report on the light-induced reduction of pyridine nucleotides by *Rhodospirillum rubrum* at the expense of the oxidation of two *p*-phenylenediamines. In the case of diaminodurol (DAD) the reduction of NAD^+ is antimycin sensitive, indicating that DAD is an electron donor at the cytochrome *b* level, whereas in the case of *N*-tetramethyl-*p*-phenylenediamine (TMPD) the NAD^+ reduction is virtually antimycin insensitive, indicating electron entry at the cytochrome c_2 level.

It remained doubtful whether ferredoxin is required for pyridine nucleotide reduction in bacteria, particularly in the case of *R. rubrum*[6]. TREBST AND BURBA have recently found a new inhibitor disalicylidenepropanediamine disulfonic acid (DSPD) of $NADP^+$ reduction in chloroplasts. It seems to be a specific inhibitor of the photosynthetic reduction of ferredoxin[7]. The present report shows that this inhibitor does

TABLE I

p-PHENYLENEDIAMINES AS ELECTRON DONORS FOR NAD^+ REDUCTION BY RHODOSPIRILLUM CHROMATOPHORES

45 min light, 8500 lux, in N_2. The reaction mixture contained in 1.5 ml: 0.1 mmole glycylglycine buffer (pH 7.4); 15 μmoles $MgCl_2$; 5 μmoles $^{32}P_i$; 0.5 μmole ATP; 30 μmoles glucose; hexokinase and particles prepared according to BALTSCHEFFSKY[16] with a final $A_{800\,m\mu}$ of 0.520.

Additions to 10 μmoles ascorbate	*NADH formed (μmoles)*	*ATP formed (μmoles)*
5 μmoles NAD^+	0	
5 μmoles NAD^+ + 0.1 μmole DAD	2.2	
5 μmoles NAD^+ + 0.1 μmole DAD + 10^{-6} M antimycin	1.0	
5 μmoles NAD^+ + 0.1 μmole DAD + 10^{-3} M DSPD	2.3	
0.1 μmole DAD		1.8
0.1 μmole DAD + 10^{-6} M antimycin		0.7
5 μmoles NAD^+ + 0.1 μmole TMPD	1.1	
5 μmoles NAD^+ + 0.1 μmole TMPD + 10^{-6} M antimycin	0.9	
5 μmoles NAD^+ + 0.1 μmole TMPD + 10^{-3} M DSPD	1.1	
0.1 μmole TMPD		3.8
0.1 μmole TMPD + 10^{-6} M antimycin		3.3

Abbreviations: DAD, diaminodurol, 2,3,5,6-tetramethyl-*p*-phenylenediamine; TMPD, *N*-tetramethyl-*p*-phenylenediamine; DSPD, disalicylidenepropanediamine disulfonic acid.

not inhibit either photosynthetic NAD^+ reduction in *R. rubrum* or photosynthetic $NADP^+$ reduction coupled to the bacterial particles *via* the ferredoxin–NADP reductase of spinach, indicating that ferredoxin is not required for bacterial NAD^+ or $NADP^+$ reduction. This is also suggested by the fact that added ferredoxin only slightly stimulates $NADP^+$ reduction.

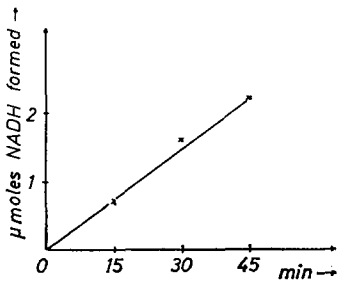

Fig. 1. Time curve of photosynthetic NAD^+ reduction by Rhodospirillum chromatophores at the expense of DAD–ascorbate (experimental conditions as in Table I).

Photosynthetic NAD^+ reduction with DAD–ascorbate as the electron donor in chromatophores from *R. rubrum* is linear with time (Fig. 1). Table I shows that ascorbate alone is not able to function as electron donor for NAD^+ reduction. NADH is obtained, however, if DAD or TMPD is added. NAD^+ reduction in the TMPD system is only slightly inhibited by 10^{-6} M antimycin, whereas the DAD system is inhibited to about 55 %. This indicates that DAD and TMPD (at the concentration of 0.1 µmole/1.5 ml used in the experiments) have different points of entry into the electron-transport chain: DAD before and TMPD after the antimycin-sensitive site. The antimycin sensitivity of NAD^+ reduction in the DAD system is roughly the same as that of cyclic ATP formation in the absence of NAD^+ with DAD as cofactor, whereas the cyclic system with TMPD is quite antimycin insensitive. TMPD is the better cofactor of cyclic photophosphorylation, whereas DAD is the better electron donor for NAD^+ reduction. A more detailed study of the cyclic system, which indicates that the point of entry is concentration dependent, is published elsewhere[8]. In chloroplast reactions too, TMPD and DAD (at a concentration of 0.2 µmole/3 ml) appear to have different points of entry into the electron-transport chain, the DAD system being coupled to ATP formation but not the TMPD system[9].

10^{-3} M DSPD, which inhibits photosynthetic $NADP^+$ reduction by chloroplasts to about 80 % (ref. 7), has no influence on NAD^+ reduction in the chromatophores, neither in the TMPD system nor in the DAD system (Table I).

A small photosynthetic $NADP^+$ reduction rate in bacteria has earlier been explained by a transhydrogenation[10] and has been ascribed recently to an energy-dependent transhydrogenase reaction[11]. Very recently, this reaction has been investigated in more detail[12]. Table II shows that another way of accomplishing $NADP^+$ reduction by chromatophores is to add ferredoxin–NADP reductase from spinach (which has transhydrogenase activity[13]). The rates of $NADP^+$ reduction in *R. rubrum* approach half the rate of NAD^+ reduction (15 µmoles/h per mg chlorophyll). Again ascorbate alone is not active; the addition of TMPD or DAD is required, DAD being the more "effective" electron donor as in NAD^+ reduction. Only the DAD system is

TABLE II

PHOTOSYNTHETIC $NADP^+$ REDUCTION BY RHODOSPIRILLUM CHROMATOPHORES

Conditions as in Table I, except that 10 μmoles P_i were added.

Additions	*NADH or NADPH formed (μmoles)*	*ATP formed (μmoles)*
to 5 μmoles NAD^+ and 10 μmoles ascorbate		
—	0	9.5
+ 0.1 μmole TMPD	0.95	9.0
+ 0.1 μmole DAD	1.3	7.2
+ 0.1 μmole DAD + 10^{-6} M antimycin	0.5	1.8
+ 0.1 μmole DAD + 10^{-3} M DSPD	1.1	7.2
to 5 μmoles $NADP^+$, ferredoxin, ferredoxin–NADP reductase from spinach and 10 μmoles ascorbate		
—	0.02	7.7
+ 0.1 μmole TMPD	0.33	6.4
+ 0.1 μmole DAD	0.53	7.0
+ 0.1 μmole DAD + $2 \cdot 10^{-6}$ M antimycin	0.10	1.7
+ 0.1 μmole DAD + 10^{-3} M DSPD	0.45	6.4
+ 0.1 μmole DAD ferredoxin omitted	0.43	5.5
+ 0.1 μmole DAD reductase omitted	0.27	5.1
+ 0.1 μmole DAD $NADP^+$ omitted	—	1.9

again rather strongly inhibited by 10^{-6} M antimycin. 10^{-3} M DSPD, which did not inhibit NAD^+ reduction, does not inhibit reduction of $NADP^+$ either. Omission of the reductase cuts the rate to about half, whereas omission of ferredoxin has only a slight effect on the rate of NADPH formation.

The lack of ferredoxin stimulation, as has already been observed earlier by NOZAKI, TAGAWA AND ARNON[10], and the indifference to DSPD, an inhibitor of ferredoxin reduction in chloroplasts[7], seem to indicate that ferredoxin is not participating in photosynthetic pyridine nucleotide reduction in chromatophores from *R. rubrum*. Two possible pathways for this reaction must be considered. (1) An unknown electron carrier with a low redox potential may be present in the chromatophores. This carrier would be reduced in light and in turn would reduce either an endogenous NAD^+ reductase or the added ferredoxin–NADP reductase from spinach. (2) The reduction may occur by means of an energy-requiring reversible electron-transport reaction from approximately the redox level of flavoprotein or *b*-type cytochrome. An initial electron acceptor at this level, capable of accepting a reducing equivalent from the light reaction, would constitute a branching point for the pathway of dark, cyclic and energy-generating electron transport back to chlorophyll and the pathway of dark, non-cyclic and energy-requiring electron transport to NAD^+ and $NADP^+$.

Non-specificity of spinach ferredoxin–NADP reductase for ferredoxin, at the electron-donor site, was already evident from the earlier observation that phytoflavin[14] is able to replace ferredoxin in spinach chloroplasts and particles from Anacystis[15].

In chloroplasts, reduction of $NADP^+$ by the DAD–ascorbate system is coupled to the formation of a stoichiometric amount of ATP (ref. 9). In chromatophores from *R. rubrum*, however, so much cyclic photophosphorylation is superimposed on the

non-cyclic system that a possible contribution to ATP formation from coupling to non-cyclic transport cannot be estimated (Table II).

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Bioenergetics Group,
Department of Plant Physiology,
University of Stockholm,
Stockholm (Sweden)

A. TREBST*
E. PISTORIUS*
H. BALTSCHEFFSKY

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* Permanent address: Abteilung Biochemie der Pflanzen, Pflanzenphysiologisches Institut der Universität Göttingen, Göttingen, Germany.

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Abnormal phytochrome spectrum in leaves

During a study of the spectral changes following dark decay of phytochrome-730 in plumules of dark-grown pea seedlings, we have previously[1] observed the formation of an absorption band at about 650 mμ. We concluded that this band must be due to the simultaneous formation of protochlorophyll ($\lambda_{max} = 650$ mμ) and phytochrome-660 in the dark. The agreement with calculated difference spectra was not very satisfactory, however. The material used in this study consisted of primary leaves with a considerable part of the third internode attached to them. In the mean time, a closer examination revealed that the leaves differ considerably from the internode sections both in their spectroscopic properties and in the kinetics of the dark decay of phytochrome-730 formed in them by irradiation with red light. Whereas the internode (apical part, hook) has little protochlorophyll and a very high concentration of phytochrome (see also ref. 2), the leaf contains relatively less phytochrome, but is