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Differential effects of DBMIB and DNP-INT on the kinetic phases of P-700 reduction

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In the presence of ferredoxin and NADP, DBMIB abolishes the fast-relaxing portion of P-700 together with the reduction of NADP. The slow-relaxing portion is inhibited at much higher concentrations. Qualitatively similar results have been observed with DNP-INT. However, its action appears to be a light-dependent process. The slow, cyclic turnover of P-700 in the presence of DCMU, ferredoxin and NADPH is inhibited by DBMIB but only slightly by DNP-INT. The data suggest that the inhibitors act at different sites of the electron-transport system.

It is well established that the reduction of P-700 after excitation by short flashes and under conditions of linear electron transport follows a polyphasic time course, but this observation has been interpreted differently by different authors [1–3]. In our previous work [3,4] we designated that fraction of P-700 which is reduced in approx. 150 μs the 'μs-component' and that which relaxes in approx. 20 ms the 'ms-component'. Only the μs-component, but not the ms-component or total P-700, could be correlated with the concomitantly measured reduction of NADP.

In a further report [5] we showed that the ms-component resisted inhibition by high concentrations of DCMU if, besides ferredoxin, NADPH was present in the reaction medium. This turnover possesses characteristics of a cyclic electron-transport system, and was thus similar to that shown in Refs. 6, 7 and 8 with other intermediates.

In the present study, we have investigated the effects of DBMIB and DNP-INT on the kinetic components of P-700 under conditions of linear (i.e. in the presence of ferredoxin and NADP) and cyclic (i.e. in the presence of DCMU, ferredoxin, NADPH) electron transport. These inhibitors are thought to block electron-transport reactions close to the plastoquinone pool. However, their actual site of action, especially that of DNP-INT, has not been established unequivocally [9–12].

The experiments were carried out with isolated broken spinach chloroplasts in a reaction medium containing 20 mM Tricine (pH 8), 100 mM sorbitol and 10 mM NaCl. P-700 turnover was measured at 700 nm by either repetitive flash spectroscopy (flash duration 10 μs, Schott RG 610 color filter) or, in experiments with DCMU, ferredoxin and NADPH by steady-state relaxation spectroscopy [13]. The flash yield of NADPH was measured by an enzymatic recycling method [14]. In continuous actinic light of saturating intensity, the reduction of NADP was measured as the time-dependent absorption increase at 340 nm. Details of these methods have been published elsewhere [13].

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Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DNP-INT, 2-iodo-6-isopropyl-3-methyl-2′,4,4′-trinitrodiphenyl ether.

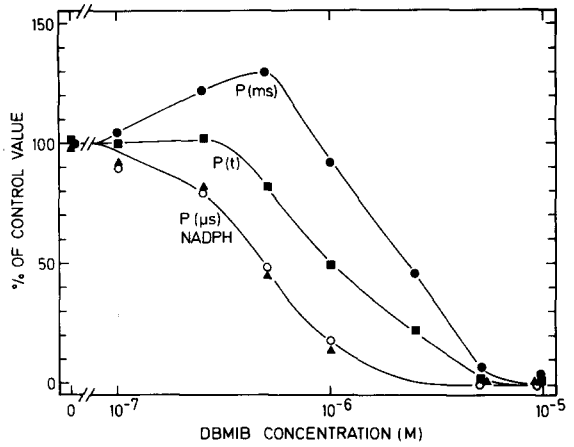

Fig. 1. Inhibition of P-700 turnover and NADP reduction by DBMIB. The samples contained chloroplast equivalent to 13 μg chlorophyll/ml, a saturating concentration of ferredoxin and 0.25 mM NADP. Measurement by repetitive flash spectroscopy. 1024 red (Schott RG 610) flashes were fired with a dark time of 300 ms. ○, P-700(μs) (control value $\Delta I/I = 1.7 \cdot 10^{-3}$); ●, P-700(ms) ($\Delta I/I = 1.1 \cdot 10^{-3}$); ■, total P-700 ($\Delta I/I = 2.8 \cdot 10^{-3}$); ▲, NADP reduction measured by enzymatic recycling. (Control value: 6.9 pmol/ml per flash.)

Fig. 1 shows the effect of increasing DBMIB concentrations on the turnover of P-700 and the concurrently measured reduction of NADP in short flashes of light. Quite clearly, the response of the μs-component is identical with that of NADP reduction, both being abolished with approx. 2 μM inhibitor, a concentration similar to that found in measurements under continuous actinic light [9]. In contrast, the ms-component first increases somewhat and is abolished only at much higher concentrations of the inhibitor.

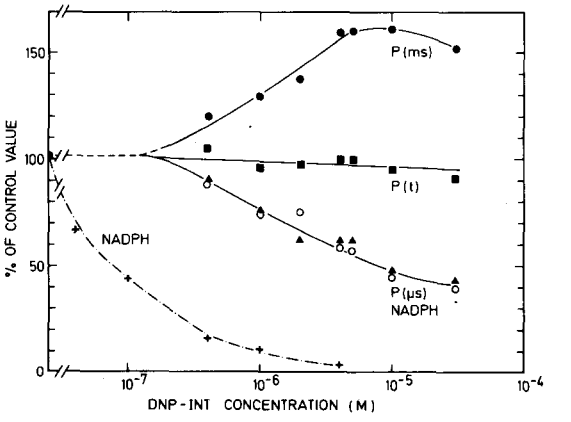

Fig. 2. Inhibition of P-700 turnover and NADP reduction by DNP-INT. Experimental conditions and symbols as in Fig. 1. Crosses: NADP reduction in continuous saturating light. (Control 102 μmol/mg Chl per h.)

As shown in Fig. 2, the simultaneous inhibition of P-700(μs) and of NADP reduction is also observed with DNP-INT. In comparison to DBMIB, however, there are two distinct differences. First, although the highest concentration of the inhibitor used is approx. 50-times that which completely eliminates linear electron transport in continuous light [10], the highest degree of inhibition observed here is only 50%. To make sure of this point, we measured the inhibition curve for NADP reduction in continuous light and included it in Fig. 2. This seems to be a rather important point, since use of this reagent in amounts that are effective in continuous light may lead to erroneous interpretations of the results when used with flashes [11]. As a possible explanation for this observation we suggest that the site of action of DNP-INT is rather inaccessible to the reagent and that light is required either to transport the inhibitor to that site or to cause structural rearrangements on the thylakoids, making that site more accessible. Although the flash intensity is high, the flash duration of 10 μs with a dark time of approx. 300 ms between flashes is apparently insufficient to this end.

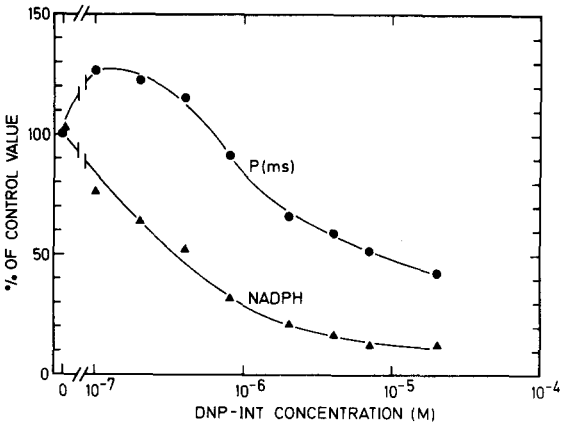

Fig. 3. Inhibition of P-700(ms) turnover and NADP reduction by DNP-INT measured by steady-state relaxation spectroscopy. The samples contained chloroplasts equivalent to 21 μg Chl/ml, a saturating ferredoxin concentration and 0.25 mM NADP. Modulation frequency of the red (Schott RG 610) actinic light was 10 cps. The absorption change of the control sample was $\Delta I/I = 2.2 \cdot 10^{-4}$; NADP reduction equivalent to 90 μmol/mg Chl per h when referred to continuous light.

The second difference is that P-700(ms) increases as before but then reaches an almost constant value. Since it is important for the interpretation of the present data to establish whether this observation is due to the light limitation of the flash or whether DNP-INT inhibits the ms-component, we repeated the experiment with a steady-state relaxation spectrometer [13]. The actinic light was modulated at 10 cps, i.e., the length of the light as well as the dark period was approx. 50 ms. Thus, if the duration of illumination is an important parameter, it could be expected that the inhibition of P-700 turnover and NADP reduction would set in at concentrations between those in continuous and flashing light. This expectation is borne out by the data in Fig. 3. First, the effective concentrations of DNP-INT are lower than in flashing light but still higher than under continuous illumination. Second, the ms-component is indeed inhibited even if at vastly higher concentration than the reduction of NADP.

The analogous inhibition by both inhibitors of the μs-component of P-700 and the reduction of NADP suggests that these reactions are interrelated, and we take the observation as further support for our previously published data and our interpretation that only the μs-component is involved in linear electron transport [3,4]. The ms-component, on the other hand, requiring appreciably higher inhibitor concentration, is obviously isolated from this pathway.

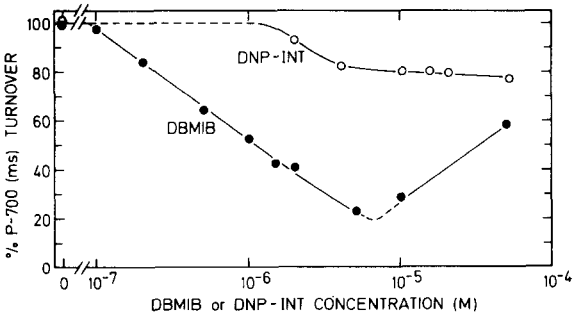

Fig. 4. Inhibition of P-700(ms) by DBMIB and DNP-INT (system: DCMU, ferredoxin, NADPH). The samples contained chloroplasts equivalent to 24.3 μg chlorophyll/ml, 10 μM DCMU, 17.3 μM ferredoxin, 0.25 mM NADPH and 10 mM Mg^{2+}. Measurements by steady-state relaxation spectroscopy. Modulation frequency of the red (Schott RG 610 filter) actinic light was 10 cps. The absorption change of the control sample was $9.1 \cdot 10^{-3}$/mg chlorophyll.

Further results were obtained with chloroplasts in the presence of DCMU, ferredoxin and NADPH. According to several reports [5–8], electron transport observed under these conditions can be ascribed to a cyclic photosystem. Presumably, NADPH reduces ferredoxin which, in turn, feeds an electron into the cycle [15]. We have shown [5] that under these conditions only the ms-component of P-700 is active, while the μs-component is inhibited. For this reason, the following measurements could be made by steady-state relaxation spectroscopy [13], ensuring furthermore that the effect of DNP-INT was not limited by light.

Fig. 4 shows that DBMIB inhibits P-700(ms) in this system effectively. Moreover, at higher concentrations turnover increases again, an observation that has previously been made with other electron-transport reactions and which has been interpreted as a shift of the site of inhibition from plastoquinol oxidation to plastoquinone reduction [9]. In contrast, DNP-INT, even at the highest concentration used, only slightly affects the measurements. These observations suggest that the inhibitors react at different sites of the electron-transport chain.

The observation that DBMIB at low concentrations inhibits P-700(ms) in the cyclic system (i.e. in the presence of DCMU, ferredoxin and NADPH) implies that plastoquinone is an intermediate of this cycle. In this respect the result resembles the observation made in the presence of ferredoxin/NADP. Presumably, electrons from NADPH enter the electron-transport chain between the sites of action of DCMU and DBMIB. According to Ref. 12 this is the plastoquinone pool. Thus, our observation is consistent with the view [16] that DBMIB at low concentrations interrupts the flow of electrons on the oxidizing side of plastoquinone (see, however, Ref. 12). At higher concentrations P-700(ms) is only little affected. It is possible that the site of inhibition shifts to the reducing side of plastoquinone by PS II [16], i.e., to a site outside the cycle.

As regards DNP-INT, Trebst et al. [10] speculatively suggested that this inhibitor interferes with the reduction of the plastoquinone pool by PS II. Haehnel and Trebst [11], in contrast, placed its site of action on the oxidizing side of plastoquinone.

Our data indicate that linear electron transport (P-700(μs), NADP reduction) is inhibited but that the cyclic system (P-700(ms)) is only little affected. Both observations can be reconciled if DNP-INT acts at a site outside the cyclic photosystem, especially between plastoquinone and PSII.

The question remains as to why P-700(ms) in the presence of ferredoxin and NADP is inhibited by DNP-INT (even if at very high concentrations) but not in the presence of DCMU, ferredoxin, NADPH.

As an explanation we suggest that the turnover of the ms-component requires a certain redox poise of the system [7]. In the presence of DCMU, ferredoxin and NADPH, the poise may be established by the reduced pyridine nucleotide. Thus, only inhibitors acting on electron-transfer outside the cyclic system (DCMU, DNP-INT) will be ineffective. In the presence of ferredoxin and NADP, the poise may be provided by the operation of linear electron transport [7]. As long as some linear electron transport remains, P-700(ms) will turn over (see Fig. 2). When its inhibition progresses, the ms-component will ultimately decline. These thoughts imply that the ms-component is not directly affected by DNP-INT but that its inhibition is due to the lowering of the redox poise.

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