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THE FUNCTION OF DIPHENYLAMINES AS MODIFIERS OF PHOTOSYSTEM II ELECTRON TRANSPORT IN ISOLATED SPINACH CHLOROPLASTS

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Summary

Diphenylamines with highly electronegative substituents are effective inhibitors of photosynthetic electron transport and photophosphorylation. They inhibit only Photosystem II- and not Photosystem I-dependent photoreductions. As judged from the missing tetramethylphenylenediamine-bypass, displacement experiments with [^{14}C]metribuzin, and measurements of oxygen evolution in trypsinated chloroplasts, diphenylamines act neither as dibromomethylisopropylbenzoquinone- nor as dichlorophenyldimethylurea-type inhibitors. All of the diphenylamines tested were found to function as ADRY-type reagents, (Renger, G. (1972) Biochim. Biophys. Acta 256, 428—439) which modify the stability of redox equivalents stored within the water-splitting enzyme system Y.

The site of inhibition of diphenylamines is assumed to be located at the reducing side of Photosystem II or the reaction center itself. The inhibitory effect could involve a modification of cytochrome *b*-559 or its surrounding. In an assay for herbicidal activity, diphenylamines showed more pronouncing effect on mono- than on dicotyledonous plants.

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Abbreviations: ADRY, acceleration of the deactivation reactions of water-splitting enzyme system Y; ANT2p, 2-(3-chloro-4-trifluoromethyl)anilino-3,5-dinitrothiophene; CCCP, carbonylcyanide-*m*-chlorophenylhydrazone; DAD, diaminodurene; DBMIB, 2,4-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone; DCMU, 3-(3,4-dichlorphenyl)-1,1-dimethylurea; metribuzin, 4-amino-6-*t*-butyl-3-methylthio-1,2,4-triazin-5-one; Mes, 4-morpholinoethanesulfonic acid; PMS, *N*-methylphenazoniummethosulfate; TMB, 3,3′,5,5′-tetramethylbenzidine; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine; Chl, chlorophyll.

Introduction

Substituted diphenylethers are widely applied as rice crop herbicides (for a recent review see Ref. 1). Their herbicidal activity is believed to be due to inhibition of photosynthetic electron transport, photophosphorylation and oxidative phosphorylation [2]. We have recently reported that, like diphenyl ethers, the structurally similar diphenylamines are potent inhibitors of photosynthetic electron transport and photophosphorylation [3]. In this respect, diphenylamines act as 'inhibitor uncouplers' as defined by Moreland and Hilton [4]. For their inhibitory activity in photosynthetic electron transport a quantitative structure activity relationship by using the Hammett electronic parameter, σ, could be established [3]. In this paper we wish to report on the site of inhibition and mode of action of diphenylamines. The results indicate that diphenylamines inhibit Photosystem II- but not Photosystem I-dependent reactions. Their site of inhibition is not identical with that of DBMIB and only for one substance (2,4,6-trichloro-2′,4′,6′-trinitrodiphenylamine) (DP 12) resembles that of DCMU. All of the diphenylamines tested were found to function as ADRY-type reagents [5—7], which modify the stability of redox equivalents stored within the water-splitting enzyme Y [8]. In some cases the ADRY-effect can only be measured in trypsinated chloroplasts, because a stronger inhibitory action overrides the ADRY-effect.

Furthermore, in a preliminary assay for herbicidal activity, diphenylamines showed more pronounced effects on mono- than on dicotyledonous plants.

Materials and Methods

Diphenylamines were obtained as described recently [3]. [^{14}C]Metribuzin was a generous gift from Dr. W. Draber, Bayer AG, Wuppertal.

Chloroplasts were prepared according to Nelson et al. [9] or Winget et al. [10] and stored until use in liquid nitrogen in the presence of 10% glycerol or 5% dimethylsulfoxide.

Trypsination of the chloroplasts was performed according to the procedure described in Ref. 11.

Photosynthetic NADP-reduction was measured at 340 nm in a Zeiss PMQ II spectrophotometer modified for illumination with actinic light at an intensity of 0.1 $W \cdot cm^{-2}$. The reaction mixture contained 20 mM Tricine/NaOH (pH 8.0), 5 mM $MgCl_2$, 17.5 μM gramicidin, 1.5 mM NADP, 5 μM ferredoxin from spinach and chloroplasts with a concentration corresponding to 7 μg/ml chlorophyll. For testing the TMPD-bypass, 50 μM TMPD was applied. For an assay of Photosystem I activity, the medium contained in addition 1 mM diaminodurene and 33 mM sodium ascorbate. pI_{50} values were determined by graphic extrapolation to 50% inhibition.

Photosynthetic ferricyanide reduction was measured at 420 nm. The reaction mixture contained 26.7 mM Tricine/NaOH (pH 8.0), 0.5 mM ferricyanide, 0.1 mM phenylenediamine, 2.5 μM gramicidin and chloroplasts with a concentration corresponding to 10 μg/ml chlorophyll. Measurement of PMS-catalyzed cyclic photophosphorylation and displacement experiments with [^{14}C]metribuzin have been performed as described recently [3].

Oxygen measurements were performed with a Clark-type electrode as described earlier [5]. In order to measure the ADRY-effect, chloroplasts were excited with repetitive short flashes (half-width 20 μs) of varying dark time, t_d, between the flashes, while for measurements of the electron transport rate via oxygen evolution the dark time between the flashes was kept constant and short compared to the rate-limiting step of the overall non-cyclic electron flow. The latter condition is sufficiently satisfied at t_d 2 ms. The reaction mixture contained chloroplasts (50 μM chlorophyll), 10 mM KCl, 2 mM $MgCl_2$ and 20 mM Mes/NaOH (pH $\leqslant$ 6.5) or Tricine/NaOH (pH $\leqslant$ 7.0).

Herbicidal activity of diphenylamines was tested with seedlings of winter wheat (*Triticum aestivum* L. cv. 'Diplomat'), winter rye (*Secale cereale* L. cv. Petkus 'Kustro'), and pea (*Pisum sativum* L. cv. 'Kleine Rheinländerin'). The plant material was grown in vermiculite at a temperature of 22°C and a light intensity of 5000 lux. A $1 \cdot 10^{-4}$ M solution of diphenylamines containing 1% dimethylsulfoxide was either applied to the soil or sprayed to the leaves every second day over a period of days. Growth was monitored over a total period of 3 weeks. Plants treated in the same way with a 1% aqueous solution of dimethylsulfoxide served as controls.

Results

All diphenylamines tested so far proved to be inhibitors of photosynthetic electron transport and photophosphorylation as well. Inhibitory activity increases with the number and the electronegativity of the substituents attached to the aromatic system which is reflected in a quantitative structure-activity relationship from the Hammett electronic parameter, σ [3]. For a close investigation of the site of inhibition and mode of action the seven most potent inhibitors out of 16 diphenylamines previously reported [3], have been examined in more detail.

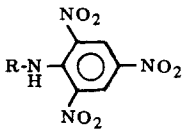
1. Inhibition of photosynthetic electron transport and photophosphorylation

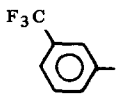
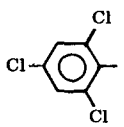
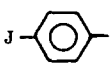
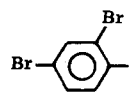
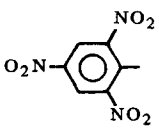
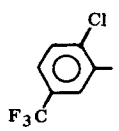
Structural formulas and pI_{50} values for inhibition of photosynthetic electron transport and photophosphorylation of the seven diphenylamines selected are listed in Table I. The numbers in Table I relate to those in the previous report [3]. The dependency on concentration for inhibition in NADP-reduction and PMS-catalyzed cyclic photophosphorylation for the most active diphenylamine compound, DP 16, is shown in Fig. 1. As demonstrated in Fig. 1, at a given concentration of DP 16 photophosphorylation is more affected than NADP reduction. In other cases, however, the opposite is true (for example DP 12, Table I).

Tischer and Strotmann [12] have recently presented evidence that extrapolated I_{50} values for zero chlorophyll concentration are identical with the binding constants. This extrapolation to zero chlorophyll concentration is shown in Fig. 2 for DP 12, DP 15, and DP 16. As can be seen, the I_{50} values for DP 15 and DP 16 are clearly chlorophyll-dependent, and binding constants, K_i, of 0.107 μM and 0.172 μM, respectively, can be calculated. In contrast, there is almost no dependency on chlorophyll concentration for the I_{50} value of DP 12 (Fig. 2).

TABLE I

STRUCTURAL FORMULAE AND pI_{50} VALUES FOR INHIBITION OF PHOTOSYNTHETIC ELECTRON TRANSPORT (ET) ($H_2O \rightarrow$NADP) AND CYCLIC PHOTOPHOSPHORYLATION (ATP) FOR SELECTED DIPHENYLAMINES (DP)



R	Abbreviation	pI_{50} (ET)	pI_{50} (ATP)
	DP 10	5.32	6.74
	DP 11	5.43	5.00
	DP 12	5.57	5.15
	DP 13	5.62	6.68
	DP 14	5.80	6.60
	DP 15	5.90	6.20
	DP 16	5.97	6.60

From the data available, Hill plots for compounds DP 13 and DP 15 could be constructed (Fig. 3). Slopes of 1.14 and 0.87, respectively, are obtained. These values are close to 1, which indicates independent binding of the inhibitor with only one site per molecule [13].

The data in Table II indicate that inhibition of photosynthetic $NADP^+$-reduction is due to an inhibition of Photosystem II. As can be seen in Table II, inhibition of photosynthetic NADP reduction by diphenylamines 10—16 can be reversed by addition of DAD/ascorbate, i.e., Photosystem I-dependent photoreduction of NADP is not inhibited. In case of DP 13 and DP 16, however, addition of DAD/ascorbate does not restore the rate of NADP reduction to the control level. There remains an inhibition of about 50% (Table II), indicating an impaired Photosystem I at high concentrations of these two compounds.

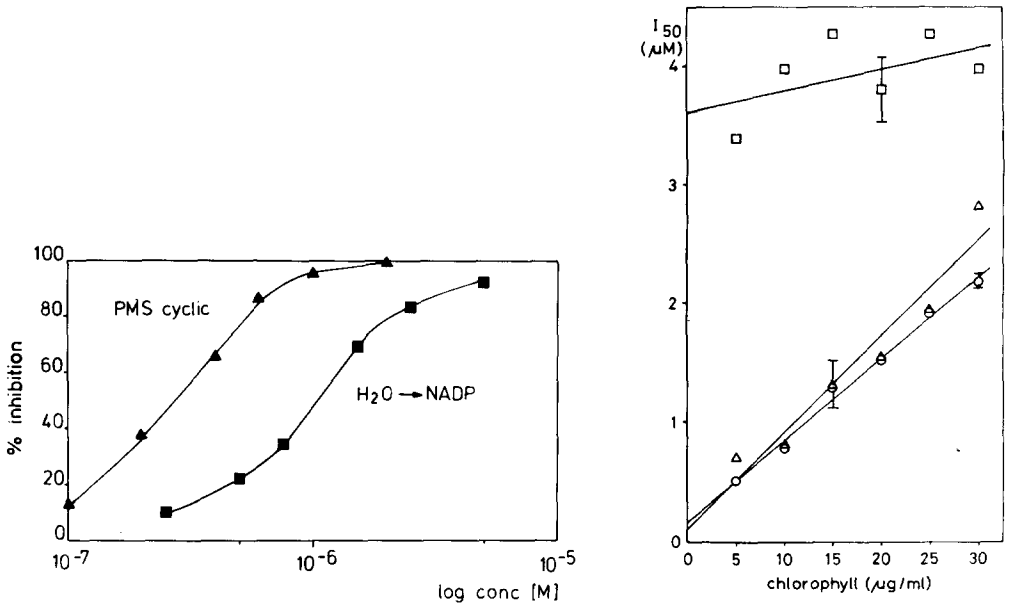


Fig. 1. Inhibition of uncoupled photosynthetic NADP-reduction ($H_2O \rightarrow NADP$) and PMS cyclic photophosphorylation by 2-chloro-5-trifluoromethyl-2′,4′,6′-trinitrodiphenylamine (DP 16). For conditions see Materials and Methods. Control rates: 179.2 µmol NADPH/mg chlorophyll per h and 716.0 µmol ATP/mg chlorophyll per h.

Fig. 2. Dependence of I_{50} values on chlorophyll concentration for DP 12 (□——□), DP 15 (△——△), and DP 16 (○——○).

The linear electron transport from water to Photosystem I can be inhibited by chemicals at different sites of the chain. DBMIB was found to impair the reoxidation of the plastoquinone pool [14], an effect which can be circumvented by a TMPD-bypass [15]. An inhibitory mechanism analogous to that of DBMIB, however, can be excluded for the diphenylamine derivatives tested in this study, because the data of Table II show that TMPD does not reverse the inhibition of $NADP^+$-reduction with water as electron donor.

The most important target for the action of herbicides on System II is the functional connection between Photosystem II and the plastoquinone pool. It has been postulated that this segment is enwrapped by a proteinaceous component [16], which is assumed to provide the binding area for this type of inhibitor. They are referred to as DCMU-type inhibitors, because DCMU is the prototype to this class of substances. Trypsination modifies the proteinaceous component, so that the electron flow into the pool is interrupted and the acceptor side of Photosystem II becomes directly accessible to exogeneous acceptors such as $K_3[Fe(CN)_6]$ [17,18]. In order to test the possibility of diphenylamines to act as DCMU-type inhibitors, the effect of these compounds on the phenylenediamine-mediated $K_3[Fe(CN)_6]$ reduction has been measured. This System II reaction is known to be blocked by DCMU [19]. The data of Table III demonstrate that DP 10 and diphenylamines 12—16 significantly inhibit the above-mentioned reaction. These results might suggest a DCMU-type inhibition mechanism. DCMU-type inhibitors are known to change the fluo-

TABLE II

INHIBITION OF PHOTOSYNTHETIC NADP REDUCTION, REVERSAL BY DAD/ASCORBATE, AND TEST OF A TMPD-BYPASS FOR DIPHENYLAMINES 10 AND 12—16

For conditions, see Materials and Methods. There was no TMPD-bypass for diphenylamines 10 and 12—16.

Compound	NADPH reduction with H_2O (control) ($\frac{\mu mol\ NADPH}{mg\ Chl \cdot h}$)	Diphenylamine concn. (M)	NADPH reduction with H_2O in presence of DP ($\frac{\mu mol\ NADPH}{mg\ Chl \cdot h}$)	Control (%)	NADPH reduction with DAD/ascorbate ($\frac{\mu mol\ NADPH}{mg\ Chl \cdot h}$)	Control (%)
DP 10	62.2	$1 \cdot 10^{-5}$	20.7	31.2	61.3	92.6
DP 12	74.4	$1.5 \cdot 10^{-6}$	17.9	24.1	70.3	94.4
DP 13	74.4	$1.5 \cdot 10^{-5}$	11.0	14.8	41.3	55.6
DP 14	68.9	$5 \cdot 10^{-6}$	10.6	15.4	63.6	92.3
DP 15	74.4	$1 \cdot 10^{-5}$	9.7	12.9	70.3	94.4
DP 16	102.1	$1 \cdot 10^{-5}$	3.1	3.0	45.9	45.0

rescence induction curve in a characteristic manner [20,21]. Preliminary experiments of the fluorescence induction in the presence of diphenylamines (Dohnt and Renger, unpublished results), however, reveal a completely different pattern, which casts doubts on a DCMU-type mechanism. To clarify this point, two types of experiment were performed: (a) displacement measurements with [^{14}C]metribuzin; and (b) measurements of the rate of oxygen evolution in trypsinated chloroplasts.

2. [^{14}C]Metribuzin displacement studies with diphenylamines

According to Tischer and Strotmann [12] the best indication of an identical inhibition site of two different inhibitors is a displacement from the chloroplast membrane of one inhibitor by the other. For this technique, either one of the two inhibitors has to be labeled by a radioactive marker. In the following experiments, [^{14}C]metribuzin has been used. Metribuzin has been recognized as an inhibitor of the DCMU type [22]. Competitive displacement experiments are shown in Fig. 4. As can be seen, increasing concentrations of diphenyl-

TABLE III

INHIBITION OF *p*-PHENYLENEDIAMINE-MEDIATED PHOTOSYNTHETIC FERRICYANIDE-REDUCTION BY VARIOUS DIPHENYLAMINES

For conditions, see Materials and Methods.

Compound	Concn. (M)	μmol $K_3[Fe(CN)_6]$/mg Chl/h		
		—DP (control)	+DP	% of control
DP 10	$5 \cdot 10^{-5}$	181.7	40.9	22.9
DP 12	$2 \cdot 10^{-5}$	184.6	38.3	20.7
DP 13	$2 \cdot 10^{-5}$	175.0	43.6	24.9
DP 15	$1 \cdot 10^{-5}$	232.5	58.9	25.4
DP 16	$2 \cdot 10^{-5}$	180.0	52.17	29.0

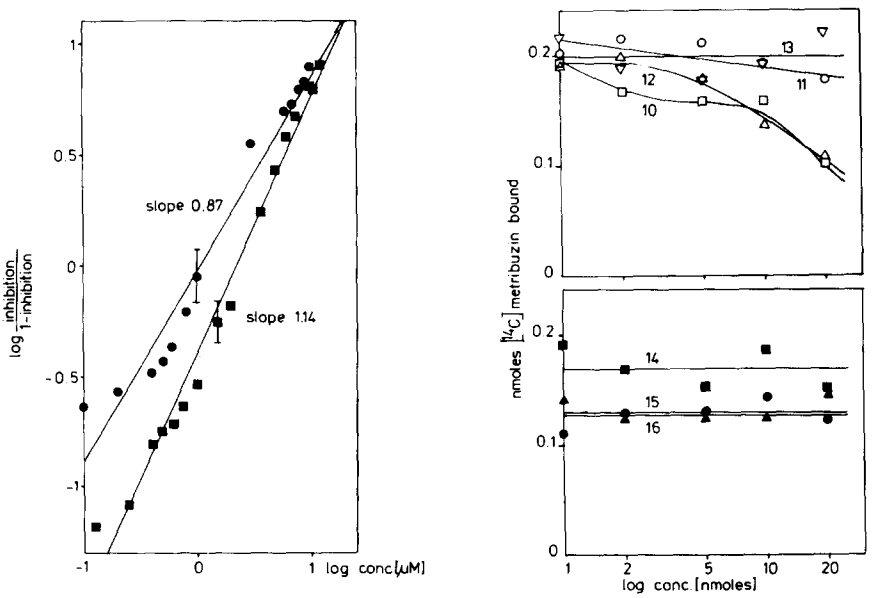


Fig. 3. Hill plot for DP 12 (■———■, $n = 16$, $r = 0.991$, $s = 0.095$) and DP 15 (●———●, $n = 13$, $r = 0.981$, $s = 0.118$)

Fig. 4. Displacement experiments of [^{14}C]metribuzin ($1 \cdot 10^{-7}$ M) by diphenylamines 10—16. For conditions, see Materials and Methods.

amines 11 and 13—16 do not displace [^{14}C]metribuzin from the chloroplast membrane. At a concentration ratio of diphenylamine versus [^{14}C]metribuzin of 100, DP 10 and DP 12 do so to a small extent. At this ratio DCMU would have removed [^{14}C]metribuzin from the chloroplast membrane almost completely [23]. Therefore diphenylamines cannot be considered as DCMU-type inhibitors.

3. Measurements in trypsinated chloroplasts

A corroboration of the above-mentioned conclusion has been obtained by measurements of the effect of trypsination. The data of Fig. 5 indicate that trypsination practically does not affect the inhibition by DP 16, while, on the other hand, the inhibition by DCMU and DP 12 is released (the stimulation of the basal electron transport by trypsin treatment is caused by its uncoupling effect). These results, which are fully consistent with the data obtained by the displacement experiments, show that DP 16 does not act as DCMU-type inhibitor, whilst DP 12 exerts an effect resembling that of DCMU. As mentioned above, it also displaces [^{14}C]metribuzin from the chloroplast membrane although to a small extent only. Another site for the attack of inhibitors is the water-splitting enzyme system Y. It can be destroyed by different treatments (for reviews see Ref. 14). A more selective modification can be achieved by ADRY-type reagents, which are known to destabilize the states S_2 and S_3 of system Y in isolated chloroplasts. At higher concentrations oxygen evolution is completely suppressed. As the diphenylamine derivatives contain acidic NH

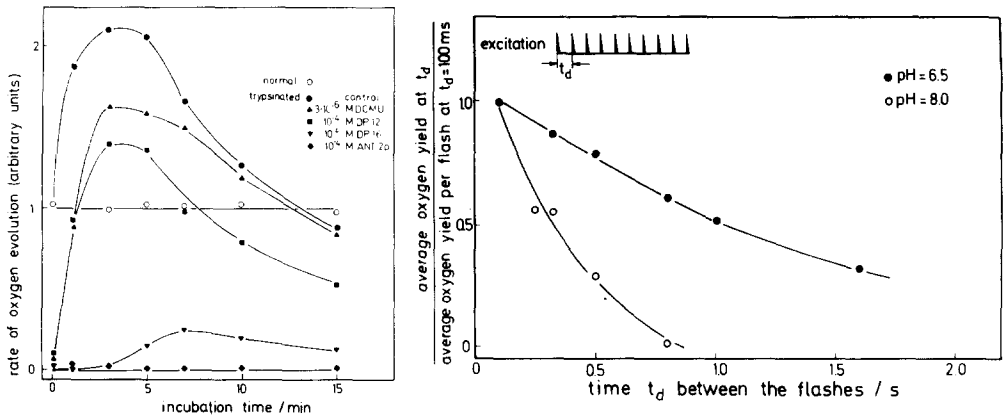


Fig. 5. Average oxygen yield per flash at t_d = 2 ms, reflecting the rate of electron transport, as a function of incubation time with trypsin in the presence of different effectors of System II in isolated spinach chloroplasts.

Fig. 6. Average oxygen yield per flash as function of the time, t_d, between the flashes in the presence of 0.3 μM diphenylamine 16 in isolated spinach chloroplasts.

groups, which were shown to be an integral part of the most powerful ADRY agents [6], the investigation of a possible ADRY effect seems to be worthwhile. In Fig. 5, it is shown that the inhibitory effect of the most potent ADRY-agent, ANT 2p, at high concentration cannot be removed by trypsin treatment. As DP 16 reacts in the same way, an ADRY-effect should be taken into consideration.

4. ADRY-type activity of diphenylamines

In order to test the possibility of an ADRY-effect, the average oxygen yield per flash as a function of the time, t_d, between the flashes has been measured at rather low concentrations of DP 16. The results, depicted in Fig. 6, show a significant decline of the average oxygen yield in a time range, where the influence due to natural deactivation reactions of S_2 and S_3 can be neglected [7]. This behavior is characteristic for the ADRY-effect.

Similar behavior, but to a weaker extent, has also been observed for the other diphenylamine derivatives (data not shown) except DP 12. Furthermore, Fig. 6 shows that the ADRY-effect is more pronounced at higher pH, which is in line with results found for other ADRY-reagents [6]. The absence of an ADRY-effect of DP 12 is not easily understandable, because of its close structural similarity to the other diphenylamines. Therefore, it appears much more reasonable to assume that the ADRY-effect is masked by the strong inhibitory effect exerted at concentrations lower than that required for the ADRY action. This assumption can be checked in trypsinated chloroplasts, which were known to lose the DCMU-type inhibition but remain sensitive towards ADRY substances [11].

The data shown in Fig. 7 confirm the anticipation that DP 12 is really able to exert an ADRY-type activity, but the effect is not observable in normal chloroplasts, because of the stronger DCMU-type inhibition. Analogous experiments

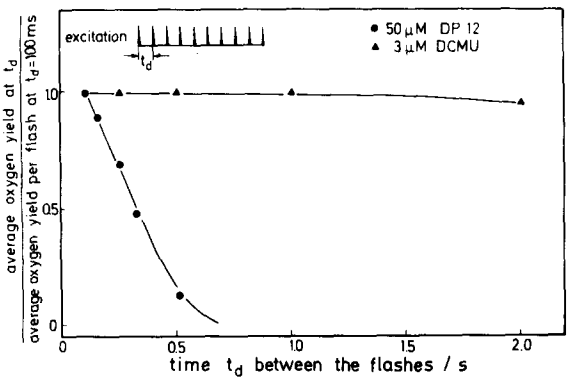


Fig. 7. Average oxygen yield per flash as function of the time, t_d, between the flashes in the presence of 3 μM DCMU or 50 μM DP 12 in trypsinated spinach chloroplasts.

have been performed for DCMU. The data in Fig. 7 do not support the previous assumption that DCMU also acts as an ADRY reagent, so that the decline of the average oxygen yield per flash with increasing time t_d [24] and the accelerated decay of S_2 and S_3 [25] in chloroplasts partially inhibited by DCMU has to be explained by another effect probably by an increase of the decay reactions of S_2 and S_3 due to an enhanced reduction degree of the electron acceptor of Photosystem II.

As ADRY-type substances like carbonylcyanide phenylhydrazones were shown to inhibit the donor side of Photosystem II at concentrations exceeding those required for the ADRY-effect [26], the inhibitory effect of diphenylamines could be caused by the same effect. In contrast to procedures such as

TABLE IV

INHIBITION OF PHOTOSYNTHETIC NADP-REDUCTION BY VARIOUS DIPHENYLAMINES IN THE ABSENCE AND PRESENCE OF THE PHOTOSYSTEM II DONOR TMB

For conditions, see Materials and Methods. In the assay system for the Photosystem II donor, the reaction mixture contained in addition $2 \cdot 10^{-4}$ M TMB and $5 \cdot 10^{-3}$ M ascorbate.

	μmol NADPH / mg Chl · h	% of control
Control	168.8	
+ $1 \cdot 10^{-5}$ M DP 12	5.8	3.4
+ TMB	27.6	16.3
Control	124.0	
+ $5 \cdot 10^{-6}$ M DP 13	17.6	14.2
+ TMB	39.8	32.1
Control	154.9	
+ $5 \cdot 10^{-6}$ M DP 14	0	0
+ TMB	28.7	18.5
Control	192.9	
+ $1 \cdot 10^{-5}$ M DP 16	5.3	2.7
+ TMB	16.0	8.3

Tris or hydroxylamine treatment leading to selective destruction of the water-splitting enzyme system, the inhibition by carbonylcyanide-*m*-chlorophenylhydrazone (CCCP) of the linear electron flow cannot be released by addition of System-II electron donors [26], e.g., diphenylcarbazide. Therefore, in order to establish whether the donor side of Photosystem II might be effected by diphenylamines, a donor system for photosystem II has been employed. As has been shown by Harth et al. [27], benzidine and its derivatives effectively can restore electron transport in hydroxylamine particles, where the water splitting enzyme system is inhibited. As can be seen in Table IV, 3,3′,5,5′-tetramethylbenzidine (TMB) restores diphenylamine-inhibited NADP reduction to only a small extent.

This small amount of restoration is probably due to the contribution of benzidines as Photosystem I donors, which has been shown to amount to about 14% of total electron flux [27]. These results indicate that the inhibitory effect of the diphenylamines closely resembles that of CCCP. It can be concluded that diphenylamines inhibit very close to the reaction centers of System II.

5. Herbicidal activity of diphenylamines

In an assay for their herbicidal activity, selected diphenylamines have been tested on two species of monocotyledon (wheat, rye) and one species of dicotyledon (pea). Spraying of the leaves with diphenylamines had no or only small effect on growth and appearance of the plants as compared to the controls. However, application of diphenylamines to the soil had serious effects on plant growth, as summarized in Table V. From all diphenylamines tested, DP 12 caused the severest responses. It led to a complete wilting of all three plant species within 10 days after application. The effect of DP 16 was similar to that of DP 12 on monocotyledons, but the dicotyledonous plant was less affected (Table V). The response of monocotyledons to DP 14 and DP 15 was less pronounced as compared to DP 16 and DP 12, and the dicotyledonous plants were not affected at all.

Finally, DP 15 behaved somewhat differently as compared to the other diphenylamines. It did not induce complete wilting as with the other compounds tested, but inhibited growth of wheat and rye, but not pea, immediately after application.

TABLE V

HERBICIDAL ACTIVITY OF SELECTED DIPHENYLAMINES ON MONO- AND DICOTYLEDONOUS PLANTS

For conditions, see Materials and Methods. Response: o, none; +, weak; ++, medium; +++, strong.

	Compound			
	DP 15	DP 14	DP 16	DP 12
Wheat (*Triticum aestivum*)	+	++	+++	+++
Rye (*Secale cereale*)	+	++	+++	+++
Pea (*Pisum sativum*)	o	o	+	+++

Discussion

The results presented in this paper indicate that diphenylamines primarily attack the System-II electron transport at the level of the water-splitting enzyme system Y via an ADRY-effect. At higher concentrations the electron transport becomes suppressed and cannot be regenerated by trypsin treatment with $K_3[Fe(CN)_6]$ as electron acceptor, in contrast to the removal of DCMU-type inhibition. A remarkable exception is diphenylamine compound DP 12, which resembles in its functional pattern DCMU with respect to the sensitivity towards trypsination of the inhibitory effect.

However, the type of fluorescence induction and the displacement experiments indicate that compound DP 12 does not act as DCMU-type inhibitor. This conclusion is further corroborated by the experiments in trypsinated chloroplasts, where DP 12 was shown to act as real ADRY-agent, whilst DCMU does not react in this way (accordingly, the DCMU-induced decay of S_2 and S_3 cannot be interpreted as an ADRY-effect: see Results). Therefore, the question arises about the functional mechanism of diphenylamines. ADRY-substances have been shown to interact with cytochrome b_{559} [28]. Accordingly, the target of diphenylamines seems to be very probably the protein moiety of cytochrome b_{559}. If one assumes additionally that cytochrome b_{559} is located close to the proteinaceous component enwrapping as an apoenzyme the functional plastoquinone molecules at the acceptor side of Photosystem II, then the attachment of the diphenylamines could be located in an area closely related to both cytochrome b_{559} and the proteinaceous component of X320 and B. If, furthermore, the binding is supposed to be dependent on the type of substitution of diphenylamines, the differences in action become understandable by slightly different localization of these substances in the above-mentioned region. A closer attachement to cytochrome b_{559} could be responsible for the predominant action as ADRY-type effector, while a stronger influence on the proteinaceous moiety of X320 and B would lead to a more pronounced inhibitory effect. Further experiments are required to clarify this hypothesis.

It has been found that ADRY-agents simultaneously act as uncouplers. Accordingly, diphenylamines exerting predominantly an ADRY-effect (DP 15 and DP 16) are anticipated to suppress via an uncoupling effect the phosphorylation more severely than the electron transport. On the other hand, diphenylamines acting as more potent inhibitors (DP 12) should be less active as uncouplers of photophosphorylation. A comparison of the data of Table I qualitatively confirms this assumption.

The results presented in this paper indicate that diphenylamines might provide a useful tool for the investigation of the architecture of System-II electron transport.

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