Bioenergetics (1973), 4, 491-505

The Modification of the Trapping Properties within the Photosynthetic Watersplitting Enzyme System Y

Gernot Renger

Max-Volmer-Institut für Physikalische Chemie der Technischen Universität Berlin

under cooperation with
Bernadette Bouges-Bocquet

Institut de Biologie Physico-Chimique, Paris 5e

and
Karl-Heinz Büchel

Bayer Forschungszentrum, 56 Wuppertal-Elberfeld

Presented at the VI International Congress on Photobiology
Bochum 21. – 25. 8. 1972

Received 12 January 1973

Summary

The modification of the trapping properties for oxidizing equivalents (holes) within the watersplitting enzyme system Y by chemicals (ADRY agents) has been investigated. It is shown:

(1) The ADRY agents decrease significantly the storage life time of the higher trapped hole accumulation states.

(2) The acidic NH- or OH-group is a functional indispensable element for substances to be able to act as ADRY agents.

(3) The trapping efficiency of the watersplitting enzyme system Y for holes is not correlated with the transmembrane electrochemical gradient.

(4) As electron donor for the discharge of the trapped holes acts an andogeneous carrier of the electron transport chain located either between the photosystems I and II respectively or on the reducing side of system I. A special electron donor is not required for the ADRY effect.

Based on these properties of the ADRY effect the nature of the traps for the storage of holes within the watersplitting enzyme system Y is discussed.

Introduction

Within the photosynthetic electron transport chain of the photoautotrophic organisms the watersplitting enzyme system Y plays a central role, since it allows the use of water as the natural electron source for the reduction of $NADP^+$. From the work of Joliot *et al* [1] and of Kok *et al* [2] two essential properties of this system Y were unequivocally proved:

(1) In respect to the cooperation of four positive charges which is required for the wateroxidation to molecular oxygen the systems Y act as functionally independent units.
(2) Each watersplitting enzyme system Y has its own trapping device which allows the accumulation of positive charges by sequential events until after the storage of four positive charges the wateroxidation takes place.

In the following these stored charges are designated as trapped holes [3].

Because of its functional role the trapped holes can be suggested as to be the chemical intermediates for the photosynthetic wateroxidation.

The reactivity of the trapped holes is unequivocally determined by the nature of their traps. In the photosynthetic watersplitting enzyme system Y two different reaction types of the trapped holes can be generally distinguished:

(a) The fast ($\tau\frac{1}{2} < 10^{-3}$ s [4, 5]) cooperative reaction of four trapped holes with water leading to the evolution of molecular oxygen

$$4\,\oplus_{trap} + 2\,H_2O \rightarrow 4\,\bigcirc_{trap} + 4\,H^+ + O_2 \qquad (1)$$

This formulation does not imply the detailed mechanism of the charge cooperation [6].

(b) The slow ($\tau\frac{1}{2} > 1$ s, s. ref. 7-9) discharge reactions of the trapped holes

$$\oplus_{trap} + e \rightarrow \bigcirc_{trap}$$

A variation of the properties of the traps by a chemical or physical effector should imply a definite change of this reaction behaviour.

Hence, information about the nature of these trapped holes should be obtainable if one could modify the traps in a well defined way. This analysis can be performed in two steps:

(1) One has to search for suitable tools for the variation of the traps.
(2) Then from the properties of these tools it should be possible to infer on the nature of the traps itself.

Recently it was found, that in chloroplasts the trapped holes can be

destabilized by a number of substances [10-13]. This effect has been called the ADRY effect [12].

Joliot *et al* [1] and Kok *et al* [2] have shown, that the states S_1, S_2 and S_3 which represent specific accumulation states of the trapped holes are characterized by different life times. The state S_1 is very stable in comparison to S_2 and S_3. Therefore, firstly the question arises, in which way the ADRY agents influence the stability of the various trapped holes accumulation states.

Secondly, in the next step of our consideration we will look for a possible mechanism of the ADRY effect and for the nature of the traps.

Materials and Methods

Preparation of Chloroplasts

The chloroplasts were prepared according to the method of Winget *et al* [14], except that 10^{-2}M ascorbate were present during the grinding of the spinach. For the storage in liquid nitrogen 5% dimethylsulfoxid was added. After thawing, the activity of the stored chloroplasts was nearly the same as for freshly prepared chloroplasts.

Reaction mixture

The reaction mixture, except that for Fig. 1 (this mixture is given in the legend of Fig. 1), contained chloroplasts (5.10^{-5}M chlorophyll), 10^{-4}M $K_3[Fe(CN)_6]$ + 10^{-4}M $K_4[Fe(CN)_6]$ as electron acceptor, 10^{-2}M KCl, 2.10^{-3}M $MgCl_2$, 2.10^{-2}M MES-buffer, pH = 6.5.

Measurements

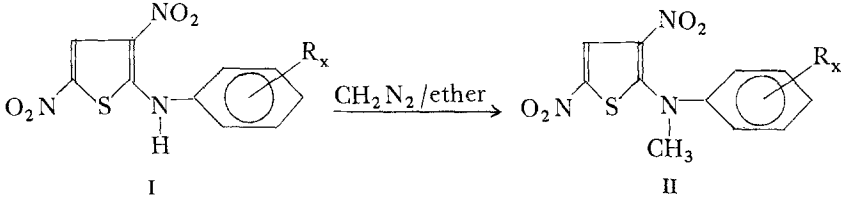
The deactivation of the active states in the watersplitting enzyme system Y described in Fig. 1 was analyzed in cooperation with B. Bouges-Bocquet in the same way as is described by Joliot *et al* [1]. For the measurements of the oxygen yield of each flash the highly sensitive oxygen polarographic method developed by Joliot [15, 16] was used.

The other oxygen measurements (Fig. 3-5) were performed with a Clark-type electrode (IL 125 B Instrumentation Laboratory Inc., Watertown) by a repetitive technique as is described in ref. 12. For the flash lamp device see ref. 17. The absorption change at 704 nm was measured with a repetitive flash spectroscopic technique similar to that published in l. c. 18. 256 signals were averaged in a FABRI-TEK 1072. The electrical bandwidth ranged from 0-30 kHz. The DC component was eliminated by a suitable compensation.

The optical path length was 1 mm, the band-width of the monitoring light (λ = 704 nm, intensity < 50 erg.cm^{-2}.s^{-1}) was 5 nm. The exciting flashes (20 μs duration) were passed through a Schott filter BG 28.

The Preparation of the N-methylederivatives of 2-anilino- 3,5-dinitrothiophenes
by Dr. K. H. Büchel

The synthesis of ADRY agents of the 2-anilino-3,5-dinitrothiophene type I has been already described elsewhere [22]. Attempts to prepare their N-methylderivatives II using CH_3J under alkaline conditions or via the sodium salts of I failed, possibly because of different tautomeric forms of structure I, which exist at pH-ranges > 8 (s. ref. 22). However, the methylation with diazomethane in ether proved to be a suitable procedure:



I II

II a R_x = 3′,4′–Cl_2 b R_x = 4′–Cl
c R_x = 3′–Cl,4′–CF_3 d R_x = 3,5–$(CF_3)_2$

Experimental:
2-(N-methyl-3′,4′-dichloroanilino)-3,5-dinitro-thiophene (IIa)

3,3 g 2-(3′,4′-dichloroanilino)-3,5-dinitro-thiophene [22] are dispersed in 50 ml of dry ether in an open flask. Under stirring 50 ml of a freshly prepared solution of diazomethane in ether are added. After 50 min of stirring the precipitate is filtered of and recrystallized from acetonitrile, yielding 2,3 g (66% of theory) of IIa, pale yellow solid, m.p. 202-204° C $C_{11}H_7Cl_2N_3O_4S$ (348,2)
Calc. :C 37.7 H 2.0 N 12.0
Found : C 38.5 H 2.1 N 12.0
UV: λ_{max} 251 nm, 400 nm (log ϵ 4.20 and 4.24)
IR: Thiopenic CH-stretching vibr. 3106 cm^{-1} asym. and sym. NO_2-stretching vibr. 1535 and 1352 cm^{-1} thiophenic and arom. C-H out of plane deformation vibr. 830 cm^{-1}, 755 cm^{-1} and 728 cm^{-1}.
In an analogous manner, the following N-methylderivatives have been prepared:

2-(N-methyl-4′-chloroanilino)-3,5-dinitro-thiophene (IIb) = NMANT 2a
IIB: m.p. 155-157° C
$C_{11}H_8ClN_3O4S$ (313.7)
Calc. : C 42.1 H 2.5 N 13.4
Found : C 42.3 H 2.6 N 13.3
UV: λ_{max} 254 nm, 398 nm (log ϵ 4.26 and 4.22)
IR: Thiopenic stretching vibr. 3104 cm^{-1}
NO_2 stretching vibr. 1535 cm^{-1} and 1348 cm^{-1}
C-H out of plane deformation vibr. 836 cm^{-1} and 720 cm^{-1}.

2-(N-methyl-3'-chloro-4'-trifluoromethyl-anilino)-3,5-dinitrothiophene (IIc) = NMANT 2p
IIc: m.p. 137-139°C
$C_{12}H_1ClF_3N_3O_4S$ (381.7)
Calc. : C 37.8 H 1.8 N 11.0
Found : C 37.7 H 1.8 N 10.8
UV: λ_{max} 248 nm, 402 nm (log ϵ 4.21 and 4.27)
IR: Thiophenic stretching vibr. 3105 cm^{-1}
No_2 stretching vibr. 1535 and 1345 cm^{-1}
C-H out of plane deformation vibr. 830 cm^{-1} and 720 cm^{-1}

2-(N-methyl-3',5'-ditrifluoromethyl-anilino)-3,5,dinitrothiophene (IId)
IId: m.p. 155-157°C
$C_{13}H_7F_6N_3O_4S$ (413.3)
Calc. : C 38.5 H 1.7 N 10.1
Found :C 37.8 H 1.8 N 9.8
UV: λ_{max} 251 nm and 394 nm (log ϵ 4.20 and 4.24)
IR: Thiophenic stretching vibr. 3110 cm^{-1}
NO_2 stretching vibr. 1535 cm^{-1} and 1340 cm^{-1}
C-H out of plane deformation vibr. 845 cm^{-1}, 728 cm^{-1} and 702 cm^{-1}

Results and Discussion

The discharge of the different trapped hole accumulation states in the watersplitting enzyme system Y by ADRY agents

Experiments to clarify the effect of the ADRY agent ANT 2p on the different accumulation states of the trapped holes were carried out in cooperation with B. Bouges-Bocquet. The obtained results are depicted in Fig. 1 (s. ref. 19). In the upper part of the semilogarithmic plot the decay of the oxygen yield Y_1 of the first flash in a short flash sequence after two preillumination flashes, which is proportional to S_3, is shown in the absence and in the presence of 10^{-6}M ANT 2p.

In the lower part of the figure the same curves are drawn for the oxygen yield Y_2 of the second flash in the sequence after one flash preillumination. This represents the decay of S_2.

By comparison one can see that the ADRY agent ANT 2p accelerates the decay of S_2 and S_3 by nearly the same degree. A similar effect for S_1 was not found (unpublished results) indicating that S_1 is strongly different in its nature compared to S_2 and S_3. This is in agreement with recent results for the redox potential of the equilibrium $S_0 \rightleftharpoons S_1$ [20]. Up to now we have discussed which accumulation states of the trapped holes are changed by the ADRY agents in their stability, i.e. we have characterized the action of the ADRY agent tool. Now it remains to gather from the physical and chemical properties of the ADRY agents on the nature of the traps.

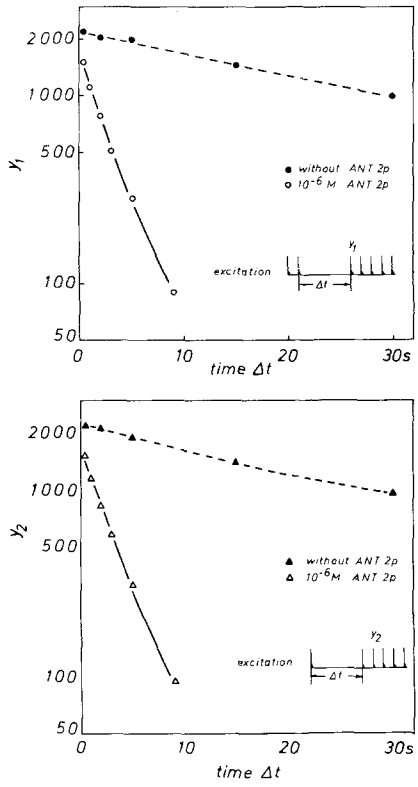


Figure 1a (top). Oxygen yield of the first flash Y_1 in a sequence of short flashes as a function of the time Δt between the flash sequence and the second flash of two preillumination flashes in chloroplasts.

The time between the two preillumination flashes was 160 ms in the presence of ANT 2p and 320 ms in its absence. Between the flashes of the sequence also a time of 160 ms was used in the presence of ANT 2p and 320 ms in its absence. Chlorophyll concentration of the chloroplasts suspension: 4.10^{-4}M. The buffer solution flowing through the cuvette contained: 10^{-4}M $NADP^+$ and 5.10^{-7}M ferredoxin as electron acceptor, 0,4 M saccharose, 10^{-2}M NaCl, 5.10^{-2}M Tris-HCl-buffer, pH = 7,5 The ANT 2p content of the buffer solution is indicated in the figure.

Other experimental conditions are as described in Materials and Methods.

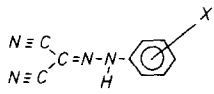
Ordinate: logarithmic.

Figure 1b (bottom). Oxygen yield of the second flash Y_2 in a sequence of short flashes as a function of the time Δt between the flash sequence and one preillumination flash in chloroplasts. Experimental conditions are as in Fig. 1a.

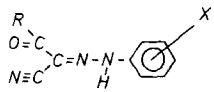
Ordinate: logarithmic.

The Properties of the ADRY Agents

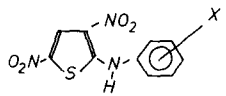
The ADRY agents so far known are compiled in Fig. 2. All of these substances are characterized by an acidic NH- or OH-group [21-23] and by the presence of delocalizable π -orbitals favoring the solubility of the negatively charged anion form in low dielectric media [24]. Because of these properties the ADRY agents act in biological systems as more or less potent uncouplers of phosphorylation and are proton translocators across artificial and biological membranes [25-27].



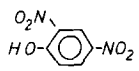
CARBONYLCYANIDE - PHENYL-HYDRAZONES



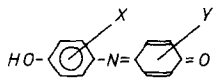
α-ACYL α-CYANOCARBONYL-PHENYLHYDRAZONES



2-ANILINO-3,5-DINITROTHIO-PHENES



NITROPHENOLS



INDOPHENOLS

Figure 2. Compilation of the ADRY agents. The ADRY-effect of the different substances has been described in: Carbonylcyanidephenylhydrazones: ref. 3, 10, 11, 42
α-Acyl αcyanocarbonylphenylhydrazones: G. Renger (unpublished)
2-Anilino-3,5-dinitrothiophenes: ref. 3, 6, 12, 30
Nitrophenols: ref. 13, 30
Indophenols: ref. 13
X, Y = Substituents of the aromatic or chinoidic rings (e.g. $-Cl$ or $-CF_3$)

The Role of the Acidic Proton for the ADRY effect

By comparison of the pK-values of 2-anilino-3,5-dinitrothiophene-derivatives and carbonylcyanidephenylhydrazones with the power of any ADRY effect induced by these substances the conclusion has been drawn that the acidic NH-group is an important functional element [12]. The role of an acidic NH-group for the activity of biological effectors was clearly shown by the use of N-methylderivatives for different uncouplers [28] and inhibitors [29]. Hence, by the use of N-methylderivatives of

ADRY agents it should be possible to decide whether the acidic proton is a functional indispensable element for substances acting as ADRY agents [30].

In Fig. 3 the effect of two N-methylderivatives of 2-anilino-3,5-dinitrothiophenes (s. Materials and Methods) on the relative average oxygen yield per flash φ (t_d) (s. ref. 12) is compared with the action of the corresponding acidic NH-group derivatives.

The results clearly show, that the ADRY effect cannot be induced by substances which do not contain an acidic proton. The next step in our consideration is to look for a possible role of the uncoupling activity of the ADRY agents.

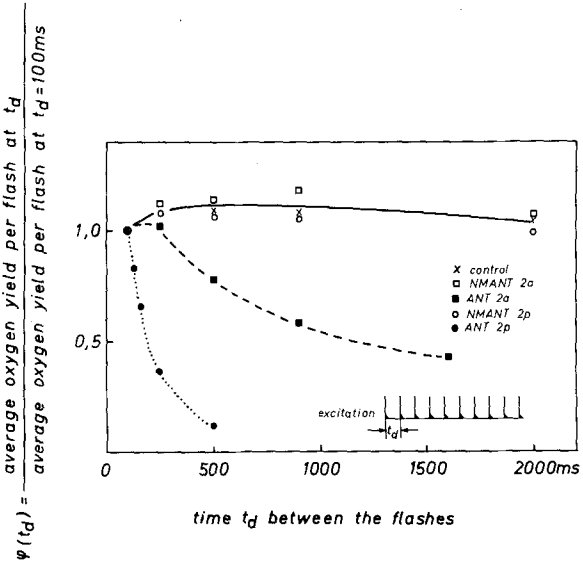


Figure 3. Relative average oxygen yield per flash $\varphi(t_d)$ as a function of the time t_d between the flashes.

The addition of the 2-anilino-3,5-dinitrothiophene-derivatives (10^{-6}M) is as indicated in the figure.

Other experimental conditions are as described in Materials and Methods.

Comparison between the Electrochemical Ion Gradient across the Thylakoid Membrane and the Stability of the Trapped Holes

It is well known, that by the light reactions a proton gradient [31, 32] and an electrical field [33] are established across the thylakoid membrane. These transmembrane phenomena could influence the stability of the trapped holes. In Fig. 4 the effect of a number of

uncouplers on the relative average oxygen yield per flash indicating the ADRY effect is compared with the stimulating effect of these agents on the Hill reaction rate which is a measure for the uncoupling activity [34]. The results show, that in respect to the ADRY effect two different

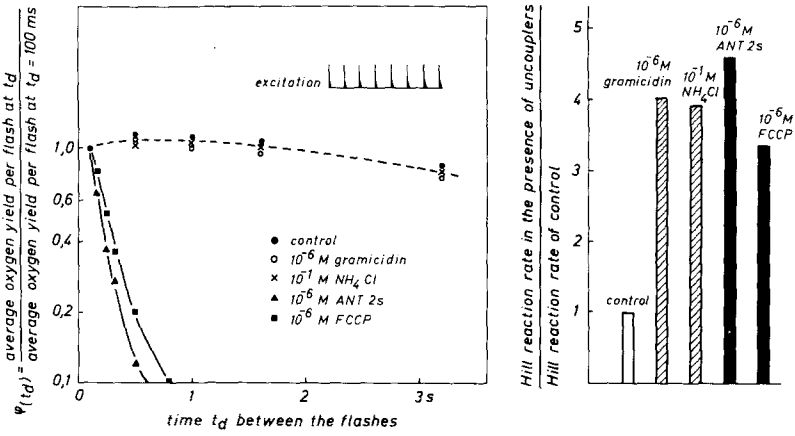


Figure 4. Comparison between the ADRY effect and the uncoupling activity for different substances.

Left: relative average oxygen yield per flash $\varphi(t_d)$ as a function of the time t_d between the flashes.

Experimental conditions are as described in Materials and Methods.

Ordinate: logarithmic.

Right: Hill-reaction rate in the presence of uncouplers related to the Hill-reaction rate of the control.

Excitation: continuous white light, saturating intensity.

Other experimental conditions as above mentioned.

Activity of control: 110 $\frac{\text{equiv. } O_2}{\text{MChl} \cdot \text{h}}$

types of uncouplers can be distinguished. One class of uncouplers (anilinothiophenes, carbonylcyanide-phenylhydrazones) is able to induce an ADRY effect, the other class (ammonium-type, gramicidin) does not destabilize the trapped holes. In this respect it is very interesting to note the results of Hardt and Malkin [35] who found that the luminescence emission of isolated chloroplasts stimulated by organic solvents (methanol) was totally inhibited by the ADRY-type uncouplers CCCP or FCCP, but was not influenced by the uncouplers gramicidin, valinomycin, NH_4Cl or atebrine. It is now well established, that the delayed luminescence of chloroplasts and algae is strongly related to the oxidizing side of System II [1, 36]. Recently we found a strong decrease

of the delayed luminescence in the presence of the ADRY agent ANT 2p [19]. Hence, the inhibition of the organic solvent triggered luminescence by the ADRY-type uncouplers is explainable in the light of the ADRY effect. The action of the different uncouplers on the triggered luminescence is in agreement with the results presented here for the destabilization of the trapped holes.

The fact, that not all types of uncouplers act as ADRY agents leads to the conclusion, that the proton gradient across the thylakoid membrane is not directly related to the trapped hole stability.

In respect to a possible influence of the electrical field on the stability of the trapped holes it has been found recently, that pure ionophores (nonactin) do not exert an ADRY effect [6]. Hence, it can be inferred, that the nature of the traps within the watersplitting enzyme system Y is not significantly influenced by the electrochemical transmembrane gradients.

The Electron Donor for the ADRY Effect

For the discharge of the trapped holes electrons are required. The electron donor for the natural discharge reactions is unknown [1, 2]. For the ADRY agent induced enhancement of the deactivation rate of the trapped holes generally two possibilities can be discussed:

(a) The ADRY agents act as irreversible electron donors which directly discharge the trapped holes.
(b) The ADRY agents build up a shunt between reduced components in the electron transport chain and the traps of the holes located in the watersplitting enzyme system.

It has been shown earlier that the first mechanism can be excluded for stoichiometrical reasons [6], so that only mechanism (b) has to be considered. From fluorescence measurements the conclusion has been drawn, that the ADRY agent ANT 2p really induces an electron cyclic flow in chloroplasts [19]. In this respect the question arises whether this cycle includes both photosystems or whether this cycle is restricted to system II. This problem can be answered by investigations of the 704 nm absorption change, which is a measure for the electrons flowing from system II through system I [37-39].

In Fig. 5 the amplitude of the 704 nm absorption change at different times t_d between the flashes is compared with the corresponding relative average oxygen yield per flash. As can be seen, the oxygen evolution dramatically decreases between 100 ms and 1 s, whereas the 704 nm absorption change is practically not changed. If on the other hand the electron flow–either in the absence (s. ref. 40) or in the presence of ADRY agents–from system II is suppressed by DCMU, the 704 nm absorption change is strongly inhibited. The small remaining amplitude

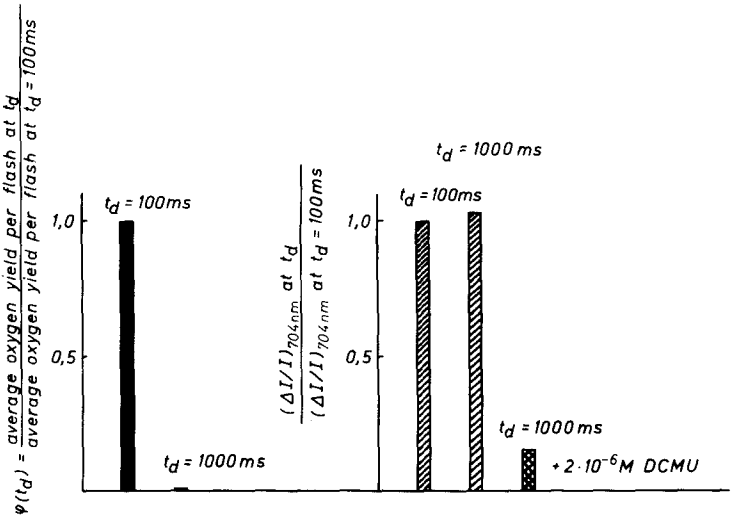


Figure 5. Comparison between the relative average oxygen yield per flash $\varphi(T_d)$ and the relative adsorption change at 704 nm as a function of the time between the flashes in the presence of 10^{-6} M ANT 210.

Experimental conditions are as described in Materials and Methods.

characterized by a very slow kinetics may be caused by another bleaching process [41].

Thus from the results of Fig. 5 an ADRY agent induced cycle around system I can be excluded. Hence, these results provide evidence for an ADRY agent mediated cycle including both photosystems. Earlier similar results were obtained for CCCP [42]. But recent experiments with system I inhibitors [43] show, that the system I activity is not an indispensable prerequisite for the ADRY effect [44]. In addition Vater [45] has shown that there exist ADRY agents which induce a cyclic electron flow including only the plastoquinone pool, but not system I.

From these results it can be inferred, that a special electron donor of the electron transport chain is not required for the ADRY effect.

The Nature of the Traps and the Mechanism of the ADRY Effect

The functional importance of the manganese for the oxygen evolving system is well known [46-48]. Hence, it has been postulated, that manganese is the central part of the traps [49-51]. Essential for the storage efficiency of these traps is the potential barrier surrounding the manganese center. The chemical components building up the barrier and their structural organization is not known, but the high sensitivity

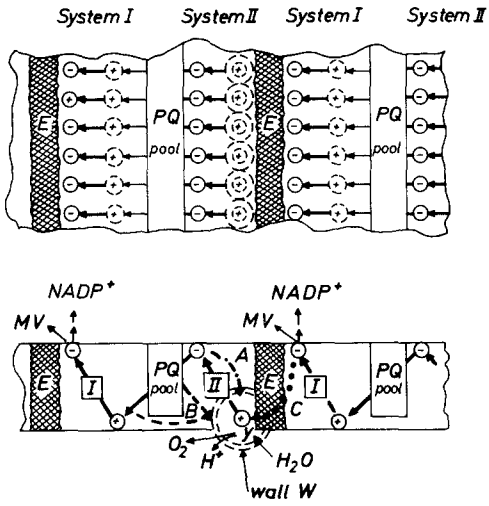


Figure 6. Scheme of a matrix array of the electron transport chains within the thylakoid membrane.

Upper part: top view.

Lower part: side view.

The negative and the positive charges represent the primary electron acceptor and the primary electron donor of both light reactions respectively.

E = energy barrier between the system I–acceptor side of one array of electron transport chains (connected by a common plastoquinone pool) with the donor side of system II of a contiguous array of electron transport chains.

W = potential wall surrounding the storage device of the holes within the watersplitting enzyme system Y.

A represents the back reaction of system II.

B symbolizes an artificial **intra-transport-chain** induced by ANT 2p. The electron donor for this cycle is located between plastoquinone and P 700.

C aymbolizes an artificial **inter-transport-chain** across the barrier E induced by ANT 2p.

The intermetiates between the light reactions and the plastoquinone pool (e.g. plastocyanin) are omitted in the scheme.

against denaturation treatments [52-55] favors the assumption of proteinaceous components. In the following the above mentioned potential barrier will be designated as the wall W. Hence, the trap properties are determined by the central manganese and the wall W.

It is assumed here that the ADRY agents modify the wall W. In respect to the electron transfer probability to the traps this can occur in two ways:

(1) The ADRY agents operate as mobile electron carriers through the wall thereby connecting the endogeneous electron source with holes stored at the manganese centers.

(2) The ADRY agents reduce the potential wall W by a fixed place mechanism.

Preliminary experiments favor a mobile carrier mechanism [44]. If one takes into account the facts, that the electron transport chains are not functionally isolated entities [56, 57] and that they are anisotropically arranged within the thylakoid membrane [58, 59] the cyclic pathways shown in Fig. 6 (s. ref. 19) can be discussed:

(a) A short cycle from the reduced primary acceptor of system II to the traps in the watersplitting enzyme system Y. This cycle is symbolized in Fig. 6 by "A". From recent experiments this cycle can be excluded as to be mainly responsible for the ADRY effect (s. ref. 19).
(b) A longer cycle from an electron donor, located between the primary acceptor of system II and P 700, to system Y. This cycle is symbolized by "B".
(c) A long cycle between the acceptor side of system I and the watersplitting enzyme system Y through an additional energy barrier E. This cycle is indicated in Fig. 6 by "C".

Based on the results presented in this paper it is argued, that both pathways ("B" and "C") are realizable by the ADRY agents.

Acknowledgements

The author wishes to thank Dr. Ch. Wolff for many fruitful discussions.
The valuable technical assistance of Miss S. Veit is gratefully acknowledged.

References

1. P. Joliot, A. Joliot, B. Bouges and G. Barbieri, *Photochem. Photobiol.*, 14 (1971) 287.
2. B. Forbush, B. Kok and M. P. McGloin, *Photochem. Photobiol.*, 14 (1971) 307.
3. G. Renger, *Physiol. Vegetale* 10 (1972) 329.
4. P. Joliot, in: *Energy conversion by the photosynthetic apparatus, Brookhaven, Sympos. Biol.*, 19 (1966) 418.
5. J. Vater, G. Renger, H. H. Stiehl and H. T. Witt *Naturwissenschaften*, 55 (1968) 220.
6. G. Renger, *Europ. J. Biochem.*, 27 (1972) 259.
7. Y. de Kouchkovsky and P. Joliot, *Photochem. Photobiol.*, 6 (1967) 567.
8. C. Lemasson, *C. R. Acad. Sci. (Paris)* 270 (1970) 250.
9. C. Lemasson and G. Barbieri, *Biochim. Biophys. Acta*, 245 (1971) 386.
10. G. Renger, *Naturwissenschaften*, 56 (1969) 370.
11. G. Renger, *Z. Naturforsch.*, 26b (1971) 149.
12. G. Renger, *Biochim. Biophys. Acta*, 256 (1972) 428.
13. J. Vater, *Biochim. Biophys. Acta* 292 (1973) 786.
14. G. D. Winget, S. Izawa and N. E. Good, *Biochem. Biophys. Res. Commun.*, 21 (1965) 438.

15. P. Joliot and A. Joliot, *Biochim. Biophys. Acta*, 153 (1968) 625.
16. P. Joliot, G. Barbieri and R. Chabaud, *Photochem. Photobiol.*, 10 (1969) 309.
17. J. Vater, *Thesis Technical University Berlin 1971.*
18. G. Döring, H. H. Stiehl and H. T. Witt, *Z. Naturforsch.* 22b (1967) 639.
19. G. Renger, B. Bouges-Bocquet and R. Delosme, *Biochim. Biophys. Acta* 292 (1973) 796.
20. B. Bouges-Bocquet, *Biochim. Biophys. Acta* 292 (1973) 772.
21. V. H. Parker, Biochem. J., 97 (1965) 658.
22. K. H. Büchel and G. Schäfer, *Z. Naturforsch.*, 25b (1970) 1465.
23. W. Draber, K. H. Büchel and G. Schäfer, *Z. Naturforsch.*, 27b (1972) 159.
24. A. Parsegian, *Nature 221* (1969) 844.
25. U. Hopfer, A. L. Lehninger and T. E. Thompson, *Proc. Natl. Acad. Sci. US*, 59 (1968) 484.
26. O. H. Le Blanc, Jr., *J. Memebrane Biol.*, 4 (1971) 227.
27. E. A. Liberman and V. P. Topaly, *Biochim. Biophys. Acta*, 163 (1968) 125.
28. K. H. Büchel, F. Korte and R. B. Beechey, *Angew. Chemie* 77 (1965) 814.
29. K. H. Büchel, W. Draber, A. Trebst and E. Pistorius, *Z. Naturforsch.*, 21b (1966) 243.
30. G. Renger, *FEBS Letters*, 23 (1972) 321.
31. A. T. Jagendorf and G. Hind, in: *Photosynthetic Mechanism in Green Plants*, Publ. 1145, *Natl. Acad. Sci.–Natl. Res. Council*, Washington 1963, 559.
32. B. Rumberg and U. Siggel, *Naturwissenschaften*, 56 (1969) 130.
33. W. Junge and H. T. Witt, *Z. Naturforsch.*, 23b (1968) 244.
34. B. Rumberg, E. Reinwald, H. Schröder and U. Siggel, *Naturwissenschaften*, 55 (1968) 77.
35. H. Hardt and S. Malkin, *Biochim. Biophys. Acta*, 267 (1972) 588.
36. J. Lavorel, *Photochem. Photobiol.*, 14 (1971) 261.
37. B. Kok, *Acta botan. neerl.*, 6 (1957) 316.
38. B. Rumberg and H. T. Witt, *Z. Naturforsch.*, 19b (1964) 693.
39. W. Haehnel, G. Döring and H. T. Witt, *Z. Naturforsch.*, 26b (1971) 1171.
40. G. Döring, G. Renger, J. Vater and H. T. Witt, *Z. Naturforsch.*, 24b (1969) 1139.
41. B. Ke, *Biochim. Biophys. Acta*, 267 (1972) 595.
42. G. Renger, *Thesis Technical University Berlin 1970.*
43. I. Brand, T. Baszynski, F. L. Crane and D. W. Krogmann, *J. Biol. Chem.*, 247 (1972) 2814.
44. G. Renger, *Biochim. Biophys. Acta* (in press).
45. J. Vater (in preparation).
46. G. M. Cheniae and I. F. Martin, *Plant Physiol.*, 44 (1969) 351.
47. R. L. Heath and G. Hind, *Biochim. Biophys. Acta*, 189 (1969) 222.
48. G. M. Cheniae and I. F. Martin, *Biochim. Biophys. Acta*, 197 (1970) 219.
49. G. Renger, *Z. Naturforsch.*, 25b (1970) 966.
50. V. M. Kutyurin, in: *Proc. 2nd Int. Congr. Photosynthesis Res., Stresa 1971.* G. Forti, M. Avron and A. Melandri (eds), Vol. 1, Dr. W. Junk Publ., The Hague 1972, p. 92.
51. G. M. Cheniae and I. F. Martin, *Plant Physiol.*, 50 (1972) 87.
52. T. Yamashita, T. Tsuji and G. Tomita, *Plant and Cell Physiol. (Tokyo)* 12 (1971) 117.
53. R. Lozier, M. Baginsky and W. L. Butler, *Photochem. Photobiol.*, 14 (1971) 323.
54. S. Katoh and A. San Pietro, *Arch. Biochem. Biophys.*, 122 (1967) 144.
55. B. R. Selman and T. T. Bannister, *Biochim. Biophys. Acta*, 253 (1971) 428.
56. U. Siggel, G. Renger and B. Rumberg, in: *Proc. 2nd Congr. Photosynthesis Res., Stresa 1971* G. Forti, M. Avron and A. Melandri (eds.), Vol. 1, Dr. W. Junk Publ., The Hague 1972 p. 753.

57. W. P. Williams, in: *Proc. 2nd Congr. Photosynthesis Res., Stresa 1971.* G. Forti, M. Avron and A. Melandri, Vol. 1, Dr. W. Junk Publ., The Hague 1972, p. 745.
58. W. Schliephake, W. Junge and H. T. Witt, *Z. Naturforsch.,* **23b** (1968) 1571.
59. G. P. B. Kraan, J. Amesz, B. R. Velthyus and R. G. Steemers, *Biochim. Biophys. Acta,* 223 (1970) 129.