BBA 46255

EVIDENCE FOR ELECTRONIC AND IONIC INTERACTION BETWEEN ELECTRON TRANSPORT CHAINS IN CHLOROPLASTS

ULRICH SIGGEL, GERNOT RENGER, HANS HENRICH STIEHL AND BERND RUMBERG
Max-Volmer-Institut für Physikalische Chemie, Technische Universität Berlin, 1 Berlin 12 (Germany)
(Received August 20th, 1971)

SUMMARY

Electron transport, phosphorylation and the absorption changes of plastoquinone and chlorophyll-a_I have been measured as a function of 3(3,4-dichlorophenyl)-1,1-dimethylurea(DCMU) concentration. The differential effect of DCMU observed is not consistent with the concept of isolated chains of electron carriers. Two types of cooperation are realized.

1. Electron exchange is possible between at least 10 chains within a common plastoquinone strand. This follows from the different sensitivity of the electron transfer reactions against DCMU with regard to short and long time excitation under uncoupled conditions.

2. All the electron transport chains of one thylakoid seem to interact by means of the internal protons which are pumped into the common inner phase by the chains and react on them by means of pH-sensitive electron transfer reactions. This is the interpretation of the different DCMU dependence of basal, coupled and uncoupled electron transport.

INTRODUCTION

In the past many laboratories have identified and investigated a number of electron carriers which mediate the transfer of electrons from water to $NADP^+$. A simplified scheme of one electron transport chain is shown in Fig. 1. The pathways of electrons have mainly been regarded, so far, as isolated chains. However, we have direct experimental evidence, that the electron transport chains interact on the level of plastoquinone which has been published in a short note in several papers[2-4], for which we would now like to present the reasoning and experimental details. Furthermore, the cooperation of electron transport chains will be discussed within the larger scope of different possibilities of interaction, proceeding from the known facts of structure and mechanism. A cooperation of different units on the level of bulk chlorophyll is already known, *i.e.* Joliot's energy transfer between Reaction centres II[5]. The next possible sites of interaction would be: the reaction centres themselves, the

Abbreviations: Chl-a_I and Chl-a_{II}, Reaction centre chlorophylls of Photosystem I and II, respectively; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; MES, 2-(*N*-morpholino) ethanesulfonic acid; PQ, plastoquinone; Tricine, *N*-tris (hydroxymethyl)methylglycine; X, unknown intermediate in the electron transport chain.

various electron carriers of the chains and the ionic atmosphere of the thylakoids. The discussion will proceed in this order. All the types of coupling may be deduced from 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) experiments.

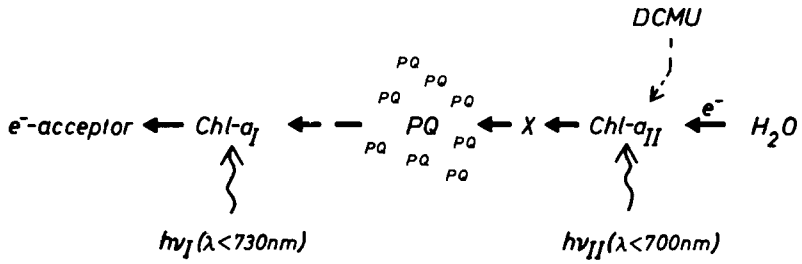

Fig. 1. Simplified scheme of one electron transport chain according to WITT *et al.*[1].

MATERIALS AND METHODS

Preparation of chloroplasts

The experiments were performed with suspensions of spinach chloroplasts which were isolated as described in ref. 6, except that 10 mM ascorbate were present during grinding. Small aliquots were frozen in liquid nitrogen (in the presence of 5 % dimethylsulfoxide) and stored until use. For all the suspensions chloroplasts from the same stock were taken. The activity of the chloroplasts frozen and thawed was as good as that of fresh samples.

Reactions mixtures

The experiments of Fig. 2 were performed at pH 6.5 and at 20°. The reaction mixture was made up of 0.1 mM ferricyanide (+ 0.1 mM ferrocyanide), for following oxygen production, or 0.1 mM benzyl viologen as electron acceptor, for following absorption changes, 5 mM 2-(*N*-norpholino)ethanesulfonic acid (MES) buffer, 10 mM KCl, 2 mM $MgCl_2$ and chloroplasts containing 10 nmoles chlorophyll per ml. 30 mM NH_4Cl was used as uncoupling agent.

The experiments of Fig. 3 were performed at pH 8 and also at 20°. The reaction mixture contained 0.5 mM ferricyanide, 10 mM *N*-tris(hydroxymethyl)methylglycine (Tricine) buffer, 50 mM KCl, 3 mM $MgCl_2$ and 1 mM ADP. For phosphorylation the medium was completed by 3 mM phosphate. Uncoupling was accomplished by 8 mM methylamine.

DCMU was added as a solution in methanol. Every sample contained exactly 1 % of methanol. DCMU, obtained from SERVA (Heidelberg), was purified by recrystallization from benzene.

Measurements

The samples were illuminated with red light of saturating intensity. The duration of the actinic flash light was 20 μsec (short flash) or 0.2 sec (long flash, sufficient to obtain stationary conditions with uncoupler). The duration of the actinic continuous light was 10 sec for the measurement of oxygen production and 2 min for ATP production.

Oxygen production was detected by means of a Clark-type oxygen electrode. If induced by short-flash light it was measured by the yield from 200 flashes, spaced 0.5 sec in time. In continuous light, and without uncoupler, 10 sec of preillumination were necessary to exclude induction phenomena.

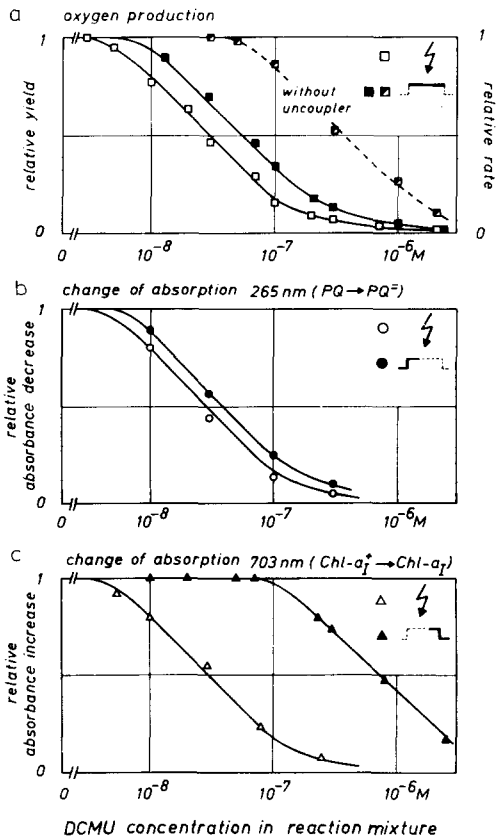

Fig. 2. Effect of DCMU on the flash yield and rate of oxygen production, respectively (a) and on the size of the absorption changes of plastoquinone (b) and chlorophyll a_I (c). Excitation with saturating short-flash light (open symbols) and saturating permanent or long-flash light (for oxygen production and absorption changes, respectively) (solid symbols). The experiments have been performed with uncoupler. The curve of oxygen production in permanent light without uncoupler is also shown (top, right). For detailed conditions see MATERIALS AND METHODS. The control values of oxygen production in permanent light were 208 and 19 mmoles electrons per sec per mole chlorophyll in the presence and absence of uncoupler, respectively. The control value of the PQ-absorbance change was 7 times larger in long- than in short-flash light.

Absorption changes of plastoquinone and chlorophyll-a_I were measured at 265 nm and 703 nm, respectively. The absorption changes at 265 nm were measured by means of the periodical flash technique[7]. 1024 signals were sampled in the case of short-flash excitation (repetition time 0.4 sec), and 100 signals in the case of long-flash excitation (repetition time 5 sec). The complete reoxidation of plastohydroquinone after every excitation was realized by a weak far-red background illumination ($\lambda = 718$ nm). The absorption changes at 703 nm were measured without sampling.

Electron flow (Fig. 3) was obtained from the linear absorption decrease at 420 nm caused by ferricyanide reduction. Concomitant ATP production was measured by means of the ^{32}P method[8].

RESULTS

We investigated the DCMU dependence of the oxygen production and absorption changes of plastoquinone and chlorophyll-a_I in saturating permanent or long-flash light as compared with saturating short-flash light. We measured the negative absorption changes of plastoquinone, indicating its reduction, after switching on the light and the positive absorption changes of chlorophyll-a_I after switching off the light, indicating the reduction of oxidized chlorophyll-a_I by reduced plastoquinone. The experiments were performed in the presence of an uncoupler and, with regard to the oxygen production in continuous light, in the absence of uncoupler. The reactions were run at pH 6.5. Uncoupling was achieved by addition of a saturating amount of NH_4Cl, so as to obtain maximal acceleration (11-fold) of the rate of oxygen production in continuous light. The relative measured quantities are depicted in Fig. 2.

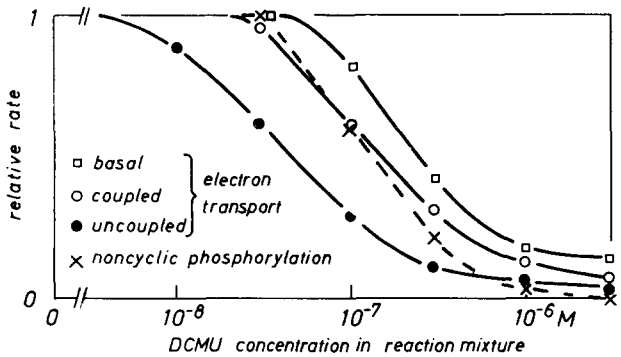

Fig. 3. Effect of DCMU on the rate of electron flow under different coupling conditions and on the rate of noncyclic ATP production at pH 8. Control values were 47, 73 and 189 mmoles electrons per sec per mole chlorophyll for basal, coupled and uncoupled electron transport, respectively, and 25 mmoles ATP per sec per mole chlorophyll for phosphorylation.

Furthermore, we measured the effect of DCMU on basal, coupled and uncoupled electron flow and on noncyclic phosphorylation at pH 8. In this case uncoupling was achieved by a saturating amount of methylamine, accelerating the basal electron flow 4-fold. The results are depicted in Fig. 3.

Experiments with uncoupler. On excitation with short flashes the oxygen yield and the size of the absorption changes of plastoquinone and chlorophyll-a_I show a nearly identical DCMU dependence with a 50 %-inhibition concentration of 30 nM. Using continuous or long-flash light for excitation, this half-value concentration is shifted to larger values in all three cases. The shift is by far the greatest in the case of the absorption changes of chlorophyll-a_I, much smaller in the case of the rate of O_2 production and still smaller for the absorption changes of plastoquinone.

Experiments without uncoupler. Concerning the rate of O_2 production (and electron flow, respectively) the half-value concentration is shifted much more than with

uncoupler. For both coupled electron flow and noncyclic phosphorylation the half-value concentration is shifted to a lesser extent than for basal electron flow.

DISCUSSION

Oxygen production and absorption changes of plastoquinone and chlorophyll-a_I are equally sensitive to DCMU if excited with short flashes (20 μsec). This can be seen from Fig. 2 and has been already stated. The flashes were short enough, so that only one electron is transferred per Centre II. According to DÖRING *et al.*[9] DCMU complexes directly with the Reaction centres II. Therefore the resulting common curve represents the relative number of active chlorophyll-a_{II} molecules.

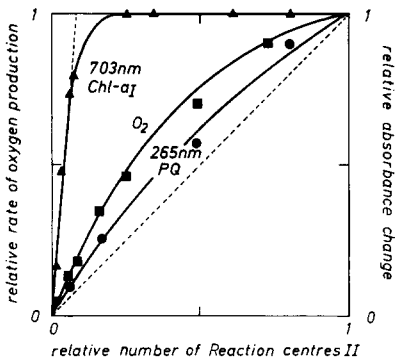

Fig. 4. The data of Fig. 2 obtained during steady state conditions are replotted as a function of the corresponding oxygen yield per short flash, representing the relative number of Reaction centres II. The initial slope of the Chl-a_I curve leads to an estimation of the number of interacting chains.

In principle, from such curves it can be concluded that the Reaction centres II exist probably as complexes of two Chl-a_{II} molecules. The number of reaction centres has to be compared with the number of binding sites for the inhibitor. In the case of independent centres the number of centres should always be equal to the number of binding sites. However, it appears that one molecule of DCMU is sufficient for the blocking of two Centres II. This structural fact may be explained by some sort of cooperation of two Chl-a_{II} molecules taking place. The details leading to this deduction are published in ref. 10.

Electron exchange between different chains via plastoquinone

It has already been stated the measured quantities do not show the same DCMU dependence if induced by short flashes or during steady state conditions. The observed shift is not consistent with the concept of independent chains. This effect indicates that an interaction of electron transport chains, by electron transfers between the carriers of different chains, takes place. A cooperation on the level of energy transfer is excluded because of the saturating light intensity and the interference of ionic gradients has been avoided by the addition of a potent uncoupler.

In Fig. 4 the relative quantities, measured in permanent or long-flash light and with uncoupler, are plotted as a function of the relative number of active Reaction centres II. The deviation from linearity indicates the existence of a cooperation. If

only the oxygen production had been measured it would be difficult to specify the mechanism of interaction. The absorption changes, however, give information as to the number of carriers which still take part in electron transfers with a reduced number of active Centres II. At a DCMU concentration where only 10 % of the Centres II are active, nearly all the chlorophyll-a_I molecules are still in action. We conclude that an electron transfer has to occur between different chains. From the initial slope (equal to 12) of the Chl-a_I curve in Fig. 4 it may be derived that at least 12 chains are interacting. One might think that the site of interaction would be the Centres I themselves, but the effect of cooperation is also evident for plastoquinone. Its concentration (in electron equivalents) is larger than that of all the other electron carriers by a factor of about 16 (ref. 4). Therefore it is reasonable to assume that the electron exchange between different chains is accomplished by this compound.

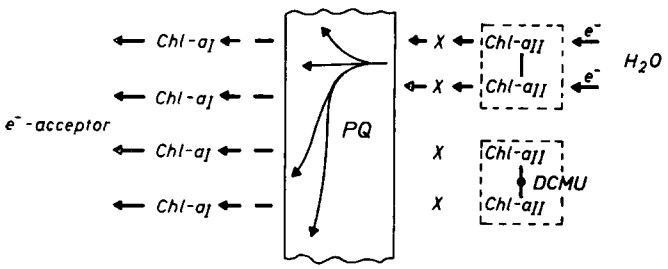

Fig. 5. Refined scheme of the electron transport system. Units of two chlorophyll-a_{II} molecules can be blocked by one molecule of DCMU. Electron exchange is possible between different chains *via* the strand of plastoquinone, connecting at least 10 chains.

Two comments should be added. Firstly, the reason for the large deviation of the relative Chl-a_I absorption changes from those of plastoquinone (PQ) seen in Fig. 4 is that about 7 electrons per Centre II accumulate in the PQ pool under normal conditions. This is seen from the factor of 7 between the amplitudes of the PQ changes in short- and long-flash light. Therefore 14 % of this maximal value of reduced PQ, produced by 10 % of Centres II, corresponds to about 1 electron per Centre II. This is approximately sufficient for the reduction of all the Chl-a_I molecules if the Centres I and II are present in equal amounts. Secondly, it should be noted that the curves for the rate of oxygen production and for the size of the absorption changes of PQ in Fig. 4 are not identical, although the rate of oxidation of reduced PQ equals that of O_2 production. The difference is due to the fact that according to the kinetic equation for the oxidation of reduced PQ derived by STIEHL AND WITT[4] the rate of oxidation is proportional to the product of the reduced and oxidized portions of PQ.

We conclude that the plastoquinone molecules of different chains form a common strand (see Fig. 5). Its function may be described as collecting electrons from the Reaction centres II and distributing them to the centres of light Reaction I.

Interaction of all the electron transport chains of one thylakoid by the protons in the inner phase

The half-value concentration of the inhibitor for permanent oxygen production is strongly shifted to greater values if the uncoupler is omitted (see Fig. 2, top). This means that, apart from the cooperation on the level of plastoquinone, there has

to exist an additional interaction. It is a reasonable assumption to associate this interaction with one of the ionic gradients across the thylakoid membrane which are well known to be formed in the absence of uncouplers. An interaction by ions might exist if one of the ions influenced the electron flow. Indeed, we have shown already that the internal protons control the electron transfer rate[11,12]. One branch of the control loop is the proton translocation into the thylakoids, which is coupled to the electron flow (see Fig. 6). The second branch is a special type of back reaction of the protons. If present in high concentration they occupy the pump stations and thereby slow down the electron flow. All the chains of one thylakoid are in contact with the same inner phase and are influenced by the protons to the same degree, that is they are coupled. If some Centres II are blocked by DCMU the influx of protons will be slower, which results in a lower internal proton concentration in the steady state. According to this lower value the electron transfer rate will be higher in the remaining active chains (chain sections). This acceleration is responsible for the constancy of the overall rate over a large range of inhibition of Centres II.

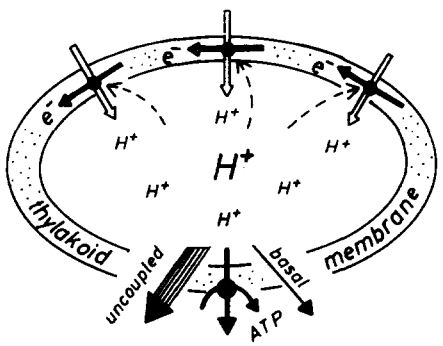

Fig. 6. Schematic representation of a thylakoid. The electron transport system is located within the membrane. Proton influxes and effluxes occur across the membrane, as indicated. The back reaction of protons on the electron flow (indicated by dashed arrows) results in an interaction of all the electron transport chains of one thylakoid.

In a measurement with uncoupler the inner proton concentration remains low because of an enhanced proton efflux[13,14]. In this case the inhibition by DCMU cannot be counterbalanced by decreased retardation of the electron flow. The sensitivity against DCMU is higher.

This interpretation is supported by the fact that noncyclic phosphorylation and the coupled electron flow are somewhat more sensitive to DCMU than the basal electron flow (see Fig. 3). This effect might be explained on the basis of the chemiosmotic hypothesis, according to which phosphorylation is coupled to an additional proton efflux out of the thylakoid. This leads to a lower proton concentration in the steady state (seen from the increased electron flow). Direct experimental evidence for this additional efflux has been given recently[15].

It might be added that the DCMU experiments alone do not allow one to specify how many electron transport chains interact on the basis of what may be generally called an energized state. However, our results on the rate control by the internal protons[11] lead us to approximately identify the stationary energized state with

the proton gradient and, accordingly, the region of interacting chains with the thylakoid.

Concluding remarks

We have seen that the different effectiveness of DCMU can be explained by different modes of interaction of electron transport chains assuming one site of action and one pathway of electrons only. The same explanation should be valid for most of the published results obtained with electron transport inhibitors, such as phenylurea derivates, quinoline-*N*-oxides and others. A satisfying general explanation for the various effects has been missing until now. Short-flash experiments have not been reported, but, instead, experiments in continuous light of low intensity are reported in refs. 16–18. The DCMU dependence of the oxygen production in both cases should only differ if energy transfer between Centres II is not negligible. The increase of effectiveness of DCMU and other inhibitors at low light intensities has been regarded as a consequence of a site of action very near to the light Reaction II. This interpretation is right, but it requires an interaction of the electron transport chains which was not stated explicitly. The differential effect according to the coupling conditions is either only manifest in the plot of measured results[19] or stated without any attempt for an explanation[20].

Recently Williams[21] also rejected the simple series model on the basis of the intensity dependent DCMU inhibition. He postulated the existence of multiple linkages between the two photosystems.

REFERENCES

1 H. T. Witt, in S. Claesson, *Nobel Symposium*, Almquist and Wiksell, Stockholm: Interscience, New York, London, Sydney, 1967, p. 261.
2 H. T. Witt, B. Rumberg and W. Junge, *19. Mosbach Kolloquium*, Springer Verlag, Berlin, Heidelberg, New York, 1968, p. 262.
3 H. T. Witt, B. Rumberg, W. Junge, G. Döring, H. H. Stiehl, J. Weikard and Ch. Wolff, in H. Metzner, *Progress in Photosynthesis Research*, Vol. 3, Tübingen, 1969, p. 1361.
4 H. H. Stiehl and H. T. Witt, *Z. Naturforsch.*, 24b (1969) 1588.
5 A. Joliot and P. Joliot, *Acad. Sci. Paris*, 258 (1964) 4622.
6 G. D. Winget, S. Izawa and N. E. Good, *Biochem. Biophys. Res. Commun.*, 21 (1965) 438.
7 H. H. Stiehl and H. T. Witt, *Z. Naturforsch.*, 23b (1968) 220.
8 S. O. Nielsen and A. L. Lehninger, *J. Biol. Chem.*, 215 (1955) 555.
9 G. Döring, G. Renger, J. Vater and H. T. Witt, *Z. Naturforsch.*, 24b (1969) 1139.
10 U. Siggel, G. Renger and B. Rumberg, *Proc. 2nd Int. Congr. Photosynthesis Res. Stresa, 1971*, in the press.
11 B. Rumberg and U. Siggel, *Naturwiss.*, 56 (1969) 130.
12 B. Rumberg, E. Reinwald, H. Schröder and U. Siggel, in H. Metzner, *Progress in Photosynthesis Research*, Vol. 3, Tübingen, 1969, p. 1374.
13 A. T. Jagendorf and J. Neumann, *J. Biol. Chem.*, 240 (1965) 3210.
14 B. Rumberg, E. Reinwald, H. Schröder and U. Siggel, *Naturwiss.*, 55 (1968) 77.
15 H. Schröder, H. Muhle and B. Rumberg, *Proc. 2nd Int. Congr. Photosynthesis Res., Stresa, 1971*, in the press.
16 J. D. Spikes, *Plant Physiol. (Suppl.)*, 31 (1956) 32.
17 G. Gingras, C. Lemasson and D. C. Fork, *Biochim. Biophys. Acta*, 69 (1963) 438.
18 S. Izawa and N. E. Good, *Biochim. Biophys. Acta*, 102 (1965) 20.
19 S. Izawa, T. N. Conolly, G. D. Winget and N. E. Good, *Brookhaven Symp. Biol.*, 19 (1966) 169.
20 M. Avron and N. Shavit, *Biochim. Biophys. Acta*, 109 (1965) 317.
21 W. P. Williams, *J. Physiol.*, 209 (1970) 13 P.