NATIVE AND ARTIFICIAL ENERGY CONSERVING SITES OPERATING IN COUPLED ELECTRON DONOR SYSTEMS FOR PHOTOSYSTEM II

E. HARTH, W. OETTMEIER and A. TREBST
Lehrstuhl Biochemie der Pflanzen, Ruhr-Universität Bochum, D 463 Bochum, W. Germany

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1. Introduction

Recent studies on ATP formation in photosynthetic electron transport in chloroplasts led to the recognition of two different energy conserving sites in non cyclic electron flow from water to NADP [1–4]. One site is connected with photosystem II and the water splitting reaction, the other with photosystem I and the proton translocation step at the functional site of plastoquinone. In addition to these native energy conserving sites artificial energy conserving sites were proposed to operate in certain coupled photoreductions by photosystem I and in cyclic photophosphorylation systems with artificial cofactors [5,6]. ATP formation in an artificial energy conservation occurs when a lipophilic hydrogen carrying electron donor is oxidized inside the thylakoid [5].

ATP formation coupled to electron donor systems for photosystem II has been described in Tris-treated chloroplasts in which specifically oxygen evolution has been inactivated [7–9] (see review [10]. We wish to report on the stoichiometry of ATP formation to electron flow ($P/_{e_2}$ ratio) in such photoreductions and its dependence on the chemical properties of the electron donor used. The results suggest that in photosynthetic NADP reduction at the expense of an artificial electron donor for photosystem II in addition to one native energy conserving site an artificial energy conserving site may or may not be induced, depending whether the oxidation of the donor liberates hydrogens or not.

Abbreviations: Cl-CCP = Carbonylcyanide-*m*-chlor-phenylhydrazone; TMPD = *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

2. Results

Benzidine has been introduced as electron donor for photosystem II in Tris-treated chloroplasts by Yamashita and Butler [7]. They also observed ATP formation coupled to NADP reduction at the expense of benzidine and other electron donors for photosystem II [7]. This has recently been confirmed by Ort and Izawa in hydroxylamine-treated chloroplasts [9].

Table 1 and fig. 1 compare the $P/_{e_2}$ ratios in photosynthetic NADP reduction at the expense of the electron donor systems benzidine/ascorbate with that of the system *N,N,N',N'*-tetramethyl-benzidine/ascorbate. The rates of electron flow from these donors to NADP in hydroxylamine treated chloroplasts approach the same value at the higher concentrations of the donor. The $P/_{e_2}$ ratio, however, is markedly different: at saturating concentrations the tetramethylbenzidine system has a ratio of 0.5 to 0.6, whereas in the benzidine system the ratio is close to 1.0 (fig. 1). Both donor systems are inhibited by DCMU as expected and are only partly sensitive to Cl-CCP, an inhibitor of oxygen evolution at the high concentration used. The uncoupler gramicidin stimulates both donor systems (table 1).

3. Discussion

Artificial electron donor systems for photosystem I may be coupled to ATP formation (in case of diaminodurene [12], indamine [13] or dichlorophenolindophenol [14] as donor) but not in others (TMPD [12,15,16] or pentamethyl-indamine [13]). Recently this was attributed to the chemical properties of

Table 1
NADP reduction and coupled photophosphorylation in electron donor systems for photosystem II in NH_2OH treated chloroplasts

Additions to 0.5 μmoles donor and 3 mM ascorbate	μmoles NADPH	μmoles ATP	P/e_2
Control without donor system	0.1	0.03	
Benzidine	1.2	1.0	0.9
Benzidine + $2 \cdot 10^{-5}$ M DCMU	0.17		
Benzidine + $3 \cdot 10^{-4}$ M Cl-CCP	0.5		
Benzidine + $5 \cdot 10^{-6}$ M gramicidin	1.6		
Tetramethylbenzidine	1.2	0.6	0.5
Tetramethylbenzidine + $2 \cdot 10^{-5}$ M DCMU	0.17		
Tetramethylbenzidine + $3 \cdot 10^{-5}$ M Cl-CCP	0.55		
Tetramethylbenzidine + $5 \cdot 10^{-6}$ M gramicidin	1.5		

Assay conditions: NH_2OH-treated chloroplast: Isolated spinach chloroplasts were suspended according to [9] in 10 μmoles NH_2OH/2 ml/mg chlorophyll at pH 7.0. After 15 min incubation in ice the solution was diluted to 40 ml and centrifuged for 1 min at 1500 *g*. The supernatant of this was centrifuged for 5 min at 20 000 *g* and the pellet suspended at pH 8 and assayed.
Photosynthetic NADPH formation was measured at 340 nm, after the chloroplasts were illuminated with 35 000 lux for 10 min at 15°C under N_2 in 3 ml containing in μmoles: tricine/NaOH buffer pH 8.0 160; $MgCl_2$ 10; ADP 10; inorganic phosphate 10 with 3.10^5 ipm P^{32}; NADP 6; ferredoxin 0.01 and chloroplasts with 200 mg chlorophyll. ATP was measured by the incorporation of radioactive phosphate according to [11].
Benzidine was obtained from Merck and tetramethylbenzidine from Eastman Kodak and recrystallized from benzene/petrolether 1:1 and then converted into the hydrochlorides by 6 NHCl, evaporated to dryness and recrystallized from methanol/acetone 1:1.

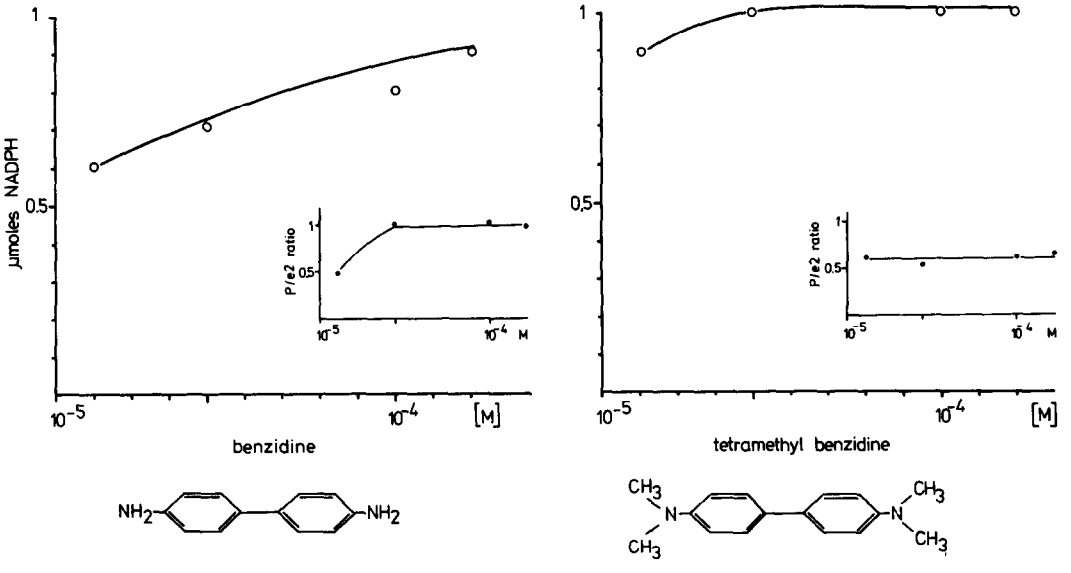

Fig. 1. Comparison of electron flow rates and P/e_2 ratio in two different electron donor systems for photosystem II. Conditions as in table 1.

the donors; those donors releasing protons upon oxidation are coupled to ATP formation, contrary to those oxidized to a radical without proton release [6,13,17]. Because plastocyanin, the endogenous electron donor for photosystem I, is probably located inside the thylakoid membrane [18], those artificial electron donors liberating protons upon oxidation at the plastocyanin site, build up a proton gradient across the membrane, whereas the others cannot. This lack of proton translocation in the TMPD donor system has recently been directly shown [19]. We have termed such ATP formation connected with proton translocation by an artificial electron donor system to be due to an artificial energy conserving site [5,6].

We would like to suggest that the different $P/_{e_2}$ ratios in electron donor systems for photosystem II, as reported here, are also due to the chemical properties of the donor used, i.e. whether the donor may or may not transport hydrogens across the membrane, when its oxidation inside the thylakoid membrane liberates protons or not. Chemically *N,N,N',N'*-tetramethylbenzidine compares with TMPD; because of the replacement of all hydrogens at the nitrogen in these two compounds by an alkylsubstitutent no hydrogens can be split off as protons upon oxidation. Whereas the TMPD system as electron donor system for photosystem I is, therefore, not coupled to ATP formation at all, a donor system for photosystem II is always coupled, because electron flow from photosystem II to photosystem I includes a native energy conserving site (proton translocation at the plastoquinone loop [20,21]). But as the results of this paper, indicate the $P/_{e_2}$ ratios in donor systems for photosystem II will be different: the tetramethylbenzidine donor system has only half the $P/_{e_2}$ ratio compared with the benzidine system. This is because the oxidation of *N*-tetramethylbenzidine cannot contribute to the proton gradient build up by the native energy conserving site, whereas oxidation of benzidine does.

In electron donor systems for photosystem II, then, a native energy conserving site might be supplemented by an artificial site, yielding together a $P/_{e_2}$ ratio of 1. It is not necessary to infere from the Same $P/_{e_2}$ ratio in the benzidine system as in non-cyclic electron flow from water to NADP that the electron donor system for photosystem II may feed in before the energy conserving site as photosystem II. Rather the native energy conserving site connected with the water splitting reaction inside the thylakoid membrane has been replaced by an artificial site connected with the hydrogen carrying property of the donor. The scheme in fig. 2 indicates the native and artificial proton releasing sites, which combined with an electrogenic photosystem comprises an energy conserving site.

The tetramethylbenzidine donor system for NADP reduction seems to reflect the true $P/_{e_2}$ ratio of the native energy conserving site connected with the proton translocation site at plastoquinone, which would yield only half the ATP formation of noncyclic electron flow. The artificial energy conserving site induced by benzidine seems to be less efficient as the native site because the benzidine system has not exactly twice the $P/_{e_2}$ ratio of the tetramethylbenzidine system.

The interpretation of these results accepted, the present view of photosynthetic electron flow vecto-

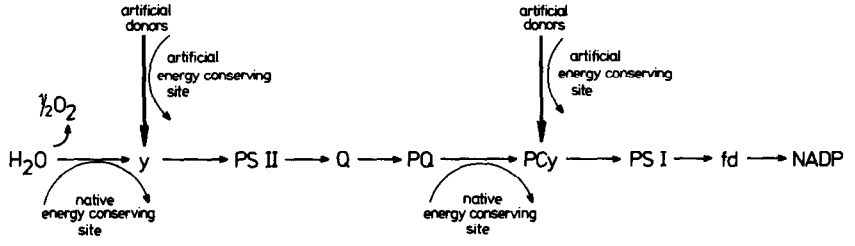

Fig. 2. Scheme of energy conserving sites in non-cyclic electron flow and in electron donor systems for photosystem I and II respectively. A native energy conserving site comprises an electrogenic photosystem and a proton releasing site inside the thylakoid membrane at the site of oxygen evolution and plastoquinone respectively. An artificial energy conserving site comprises the same electrogenic photosystem, but an artificial proton releasing site when the artificial electron donor liberates protons upon oxidation inside.

rial across the thylakoid membrane is strengthened [22,23] (see review in ref. [24]).

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