Inhibition of photosynthetic oxygen evolution by protonophoric uncouplers

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Abstract

The protonophoric uncouplers carbonyl cyanide m-chlorophenylhydrazone (CCCP), 2,3,4,5,6-pentachlorophenol (PCP) and 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole (TFFB) inhibited the Hill reaction with K₃[Fe(CN)₆] (but not with SiMo) in chloroplast and cyanobacterial membranes (the Iₕ values were approx. 1–2, 4–6 and 0.04–0.10 μM, respectively). The inhibition is due to oxidation of the uncouplers on the Photosystem II donor side (ADRY effect) and their subsequent reduction on the acceptor side, i.e. to the formation of a cyclic electron transfer around Photosystem II involving the uncouplers as redox carriers. The relative amplitude of nanosecond chlorophyll fluorescence in chloroplasts was increased by DCMU or HQNO and did not change upon addition of uncouplers, DBMIB or DNP-INT; the HQNO effect was not removed by the uncouplers. The uncouplers did not inhibit the electron transfer from reduced TMPD or duroquinol to methylviologen which is driven by Photosystem I. These data show that CCCP, PCP and TFFB oxidized on the Photosystem II donor side and their subsequent reduction on the acceptor side, i.e. to the formation of a cyclic electron transfer around Photosystem II involving the uncouplers as redox carriers. The relative amplitude of nanosecond chlorophyll fluorescence in chloroplasts was increased by DCMU or HQNO and did not change upon addition of uncouplers, DBMIB or DNP-INT; the HQNO effect was not removed by the uncouplers. The uncouplers did not inhibit the electron transfer from reduced TMPD or duroquinol to methylviologen which is driven by Photosystem I. These data show that CCCP, PCP and TFFB oxidized on the Photosystem II donor side are reduced by the membrane pool of plastoquinone (Qₚ) which is also the electron donor for K₃[Fe(CN)₆] in the Hill reaction as deduced from the data obtained in the presence of inhibitors. Inhibition of the Hill reaction by the uncouplers was maximum at the pH values corresponding to the pK of these compounds. It is suggested that the tested uncouplers serve as proton donors, and not merely as electron donors on the oxidizing side of Photosystem II.

Abbreviations: ADRY – acceleration of the deactivation reactions of the water-splitting enzyme system Y; ANT2p–2-(3-chloro-4-trifluoromethyl)anilino-3,5-dinitrothiophene; CCCP – carbonyl cyanide m-chlorophenylhydrazone; DBMIB – 2,5-dibromo-3-methyl 6-isopropyl-p-benzoquinone; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DNP-INT – 2-iodo-6-isopropyl-3-methyl 2',4,4'-trinitrodiphenyl ether; DPC – 1,5-diphenylcarbazide; DPIP – 2,6-dichlorophenolindophenol; FCCP – carbonyl cyanide p-trifluoromethoxyphenylhydrazide; FeCy – potassium ferricyanide; HQNO – 2-n-heptyl-4-hydroxyquinoline N-oxide; (MN)₄ – the tetranuclear Mn cluster of water oxidizing complex; P680 – photoactive Chl of the reaction center of Photosystem II; PCP – 2,3,4,5,6-pentachlorophenol; PS – photosystem; Qₐ and Qₐ – primary and secondary plastoquinones of PS II; Qₐ and Qₐ – plastoquinone binding sites in the cytochrome b/f complex; Qₚ – membrane pool of plastoquinone; SiMo – sodium silicomolybdate; TMPD – N,N',N'-tetramethyl-p-phenylenediamine; TFFB – 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole; WOC – water oxidizing complex; Y₂ – tyrosine-161 of the Photosystem II D1 polypeptide
Introduction

In all O₂-evolving photosynthesizing organisms the key steps of water cleavage take place within the PS II complex that acts as a water-plastoquinone oxidoreductase (for recent reviews, see Debus 1992; Renger 1993). The overall reaction comprises a sequence of univalent oxidation steps at a manganese containing functional unit referred to as the water oxidizing complex (WOC). A redox active tyrosine of polypeptide D1 (symbolized by Y₂ and identified as Tyr 161) mediates the electron transfer from the WOC to P680⁺. The different redox states of the WOC are symbolized by Sₙ, where the index n represents the number of stored oxidizing equivalents. O₂ is released after attaining redox state S₄ which rapidly converts into S₀. The redox states S₂ and S₃ relax to S₁ via reduction by endogenous reductants in the time domain from seconds to a few minutes, whereas S₀ becomes slowly oxidized to S₁ by the oxidized form Y₂OX of the redox active tyrosine of polypeptide D2 (for a recent detailed analysis of these reactions, see Messinger et al. 1993).

The reaction pattern can be modified selectively by different types of exogenous agents: (i) small hydrophilic reductants like NH₂NH₂ and NH₂OH leading to 'super-reduced' states S₋₁ and S₋₂ (see Messinger and Renger 1993 and refs. therein), (ii) lipophilic electron donors like tetraphenylboron that compete effectively with water for oxidizing redox equivalents (Erixon and Renger 1974), and (iii) ADRY substances causing an accelerated decay of redox states S₂ and S₃ of the WOC (Renger et al. 1973b; Hanssum et al. 1985).

The latter type reactants comprise a number of chemicals with different structures like anilinothiophenenes (Renger et al. 1973b; Hanssum et al. 1985), phenylhydrazone derivatives (Etienne 1974), nitrophens (Renger 1972), indophenols (Vater 1973) and diphenyl amines (Oettmeier and Renger 1980). As a common structural element, all ADRY agents so far identified contain an acidic OH- or NH-group and act as potent protonophoric uncouplers. In general, ADRY agents exert different effects depending on their concentration used. At sufficiently low concentrations, these substances specifically accelerate the S₂ and S₃ decay without any effect on the electron transfer from Y₂ to P680 (Renger et al. 1989). Stoichiometric considerations based on measurements of the average oxygen yield under repetitive flash excitation led to the conclusion that ADRY agents act as mobile catalysts of a cyclic electron flow giving rise to S₂ and S₃ reduction by endogenous electron carriers (Renger et al. 1973b, Hanssum et al. 1985). Interestingly, the most powerful species even act at substoichiometric amounts of about 1 ADRY molecule per 10 PS II complexes (Hanssum et al. 1985). The idea of an ADRY agent induced cycle is supported by the finding that the electron flow to exogenous electron acceptors like methylviologen (Renger et al. 1973b) or DPIP (Packham and Barber 1984) becomes diminished. At higher concentrations additional effects arise as reflected by several phenomena: (a) light-induced oxidation of carotenoids (Velthyus 1981) and of chlorophyll a (Yamashita et al. 1969), (b) transformation of cytochrome b-559 from its high to its low potential form (Cramer and Whitmarsh 1977; Maroc and Garnier 1979), and (c) inhibition of electron flow in chloroplasts and cyanobacterial membranes under light-saturating conditions (Renger 1975; Barsky et al. 1991a).

It has been shown that ADRY agents are redox-active reducing S₂ and S₃ in normal chloroplasts (Renger et al. 1973b; Hanssum et al. 1985) and Y₂OX in samples deprived of their oxygen evolution capacity (Renger and Reuter 1982; Ghanotakis et al. 1982). The oxidized forms of ADRY agents become reoxidized by endogenous redox components probably by plastohydroquinone at the level of Q₀ (Samuilov and Barsky 1993).

The goal of this work is to study inhibitory effects of the phenylhydrazone derivative CCCP on the electron transport and to compare them with those elicited by two other protonophoric uncouplers of benzimidazole and phenolic-type compounds.

Materials and methods

Chloroplasts from the leaves of 12 to 15 day old pea or wheat seedlings were isolated as reported previously (Barsky et al. 1991a,b). Thylakoid membranes from cyanobacterium Anacystis nidulans were prepared as in Barsky et al. (1988). Chromatophores were isolated from Rhodospirillum rubrum as described by Isaev et al. (1970). In the experiments, the chloroplasts were incubated in medium I containing 0.4 M sucrose, 35 mM NaCl, 50 mM Tricine-KOH (pH 7.8), or medium II containing 0.5 M sucrose, 0.5 M sodium citrate, 50 mM NaH₂PO₄ (pH 7.0), 20 mM KCl and 2 mM MgCl₂; thylakoids — in medium II without MgCl₂; the chromatophores — in a medium containing 0.25 M sucrose and 50 mM Tris-HCl (pH 7.6).