Energy transfer inhibition in photosynthesis by anthraquinone dyes

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1. INTRODUCTION

When purifying pyruvate kinase from yeast, Haeckel et al. [1] observed that the enzyme cochromatographed on a gel filtration column with blue dextran which is normally used as a marker of the void fraction. It was shown that the dye Cibacron Blue 3 G-A (= Reactive Blue 2, formula see table 1) which is covalently linked with its triazinyl moiety to dextran, is responsible for binding of pyruvate kinase. In the meantime a variety of kinases, dehydrogenases and other enzymes were found to interact with blue dextran (review in [2]), and this property was used for affinity chromatography. Usually the free dye binds even stronger and inhibits the enzyme reactions in competition to the involved adenine or pyridine nucleotide, respectively. More recently, Bornmann and Hess [3] reported that mitochondrial ATPase from yeast is also inhibited by Cibacron Blue 3G-A and related compounds. The structure–activity analysis demonstrated that the anthraquinone moiety as well as the neighbouring phenyl group and a hydrophobic substituent play a role in the interaction with diverse proteins. Boos and Schlimme [4] found that a series of anthraquinone derivatives inhibited oxidative phosphorylation of submitochondrial particles from rat liver mitochondria as well as the mitochondrial adenine nucleotide carrier. In the present communication the effect of those compounds on photophosphorylation is investigated. Since proton translocating reversible ATPases from different sources exhibit essentially the same structural and functional properties, interaction of these dyes with the chloroplast ATPase could be expected. Among the studied derivatives some effective energy transfer inhibitors of the chloroplast system were found, the most powerful being Reactive Blue 2. The results show that the site of interaction is the CF_1 part of chloroplast ATPase.

2. MATERIALS AND METHODS

2.1. *Anthraquinone dyes*

The formulas of the employed compounds are figured in table 1. The compounds were either commercially available or gifts from Bayer AG., Leverkusen (compounds 6 and 9) and used without further purification.

2.2. *Assay systems*

Chloroplasts were prepared from spinach leaves as in [5]. Cyclic phosphorylation was measured as in [6] in a medium containing 25 mM Tricine buffer (pH 8.0) 50 mM NaCl, 5 mM $MgCl_2$, 5 mM ^{32}P-labeled P_i, 200 μM ADP, 50 μM PMS and chloroplasts equivalent to about 25 μg chlorophyll/ml. The reaction time was 15 s, the light intensity 450 $W \cdot m^{-2}$ (white light) and the temperature 20°C.

Electron transport from water to methylviologen was assayed in the same medium except that PMS was replaced by 0.5 mM methylviologen and 1 mM KCN was added. Methylviologen reduction was measured by oxygen uptake using a Clark type electrode. The light intensity was 350 $W \cdot m^{-2}$ (red light, Filter RG 630, Schott), the temperature

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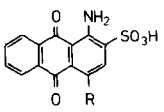
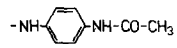
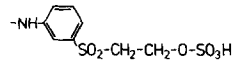
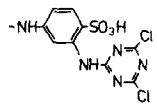
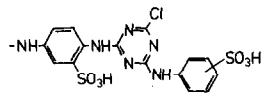
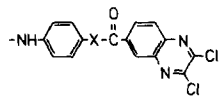
Table 1

Inhibition of cyclic photophosphorylation by anthraquinone derivatives (figures in brackets indicate the colour index numbers)



	-R		I_{50} (μM)
1	-H	1-Amino-anthraquinone-2-sulfonic acid	150
2		Acid Blue 25 [62055]	28
3		Acid Blue 40 [62125]	130
4		Acid Blue 41 [62130]	78
5		Uniblue A	53
6		Reactive Blue 19 [61200]	21
7		Reactive Blue 4 [61205]	11
8		Reactive Blue 2 [61211]	4
9	 Partial structure (patent Bayer AG)	Reactive Blue 29	18

20°C, Light-triggered ATPase was assayed as in [7] and ATP hydrolysis by isolated CF_1 as in [8].

3. RESULTS AND DISCUSSION

Table 1 shows I_{50} values for the inhibition of PMS-mediated photophosphorylation by 9 different compounds derived from 1-amino-anthraquinone-2-sulfonic acid by substitutions at position C-4. Substitution by an anilino group or its derivatives yields fairly good inhibitors (compounds 2-5).

The inhibitory power is markedly increased if an additional sulfonate group is introduced as in compounds 6–8. The most potent inhibitors are those

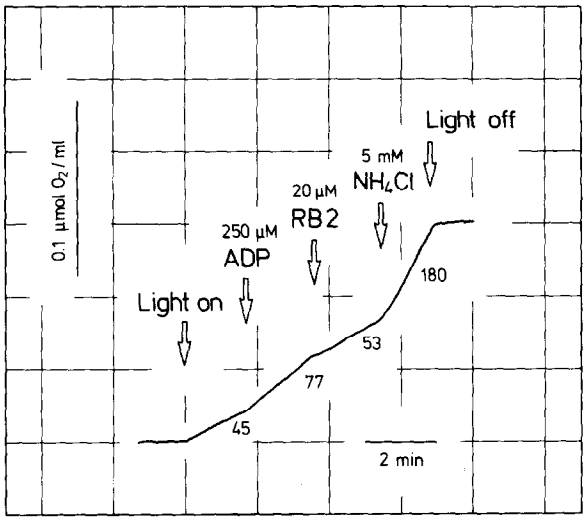


Fig.1. Inhibition of coupled electron transport by RB2 and release of inhibition by uncoupling.

which bear a triazinyl or a chinoxalinyl ring (compounds 7–9). Interestingly, among 2,4,6-substituted triazines there are several potent herbicides (e.g., Atrazin) which are known to inhibit photosynthetic electron transport in the PSII region [9,10]. The direct effect of the here employed triazinyl dyes on electron transport is, however, marginal (see fig.2). Moreover unlike anthraquinone-2-sulfonic acid [11], none of the dyes is an electron acceptor in photosynthesis. Although the experimental material is too small to draw conclusions on structure–activity relationships, it is evident that besides the anthraquinonoide chromophor additional imino-bridged aromatic ring systems contribute to the inhibitory power of these compounds in photophosphorylation.

Subsequent studies on the mechanism of inhibition have been performed with Reactive Blue 2 (compound 8). Figure 1 shows in a trace of oxygen consumption that RB 2 inhibits coupled electron transport to the rate of basal electron transport and that this inhibition is released by the addition of an uncoupler. Accordingly its behaviour is that of an energy transfer inhibitor. This is analyzed in detail in an experiment shown in fig.2. It demonstrates that basal electron transport is virtually not affected and the effect of RB 2 in the indicated concentration range on uncoupled electron transport is likewise weak. The I_{50} for coupled electron

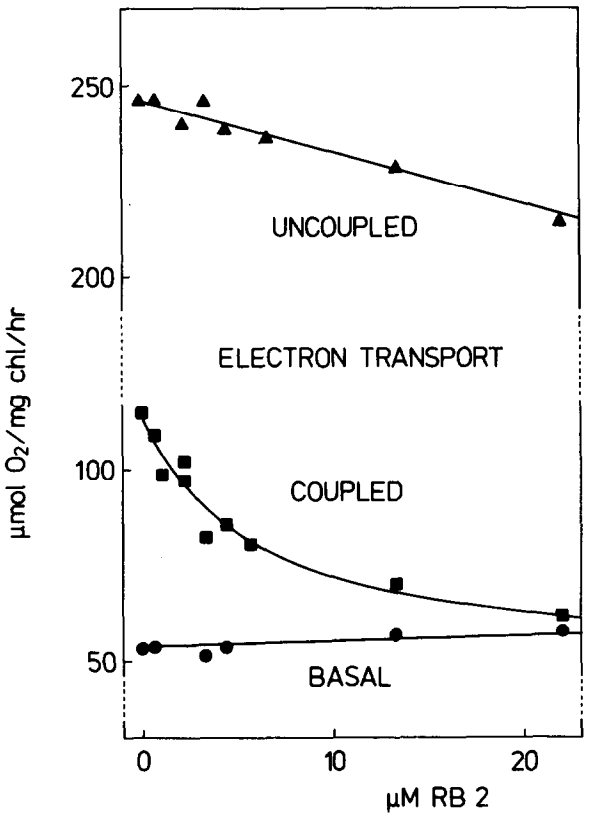


Fig.2. Effects of RB2 on basal, coupled and uncoupled electron transport. Basal electron transport was measured in the absence of P_i but presence of ADP. Uncoupled electron transport was assayed in the same medium but the used chloroplasts were preparatively uncoupled by removal of CF_1 as described in [10].

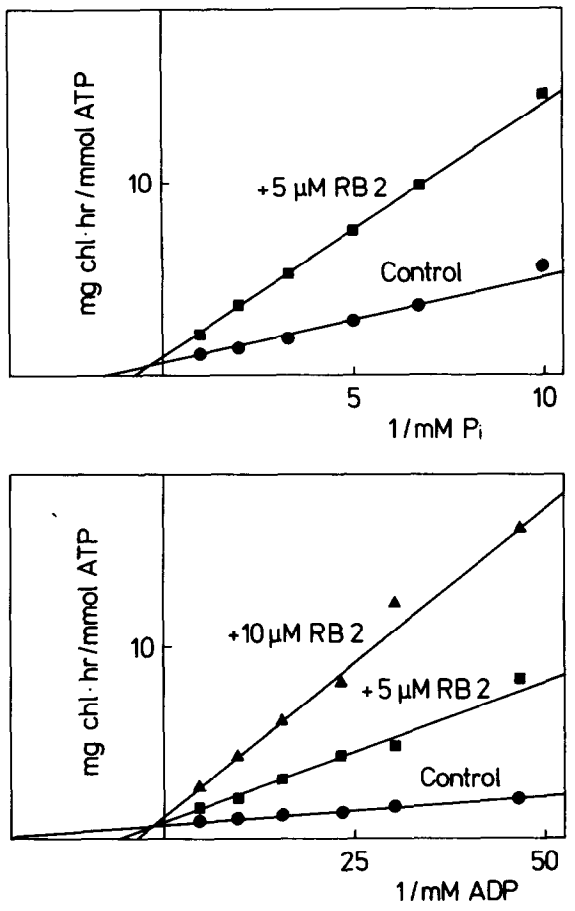


Fig.3. Effect of RB2 on cyclic photophosphorylation as a function of P_i and ADP concentration, respectively.

transport (5 µM) is in good agreement with the I_{50} in cyclic phosphorylation as shown in table 1. It is needless to mention that non-cyclic phosphorylation in a methylviologen system is also inhibited in parallel to coupled electron transport.

In fig.3 inhibition is studied by analyzing the effect of RB 2 on steady state kinetics of phosphorylation. The Lineweaver–Burk plots reveal no clear-cut conventional type of inhibition. By variation of the substrate ADP as well as P_i, mixed types in between competitive and non-competitive inhibitions are observed. Most of the known energy transfer inhibitors are clearly non-competitive, so far only some 3′-modified ADP analogs were reported to be competitive to ADP on the active site of the chloroplast ATPase [7,12,13].

Figure 4 shows the effect of RB 2 on light-triggered ATP hydrolysis. In order to induce ATPase activity of chloroplasts in the dark, pre-illumination of the thylakoid membranes in the presence of a thiol compound is required. Energy-induced activation of ATPase is related with release of tightly bound ADP from CF_1 [14–16]. To discriminate between the effects of RB2 on enzyme activation and the catalytic process, respectively, the inhibitor was added either before or after the light pre-treatment. The results in fig.4 demonstrate that in both cases the same inhibition curves are obtained, indicating that it is the ATPase reaction itself which is affected by RB 2. This is further corroborated by the fact that release of tightly bound ADP from membrane-associated CF_1 is not affected by the

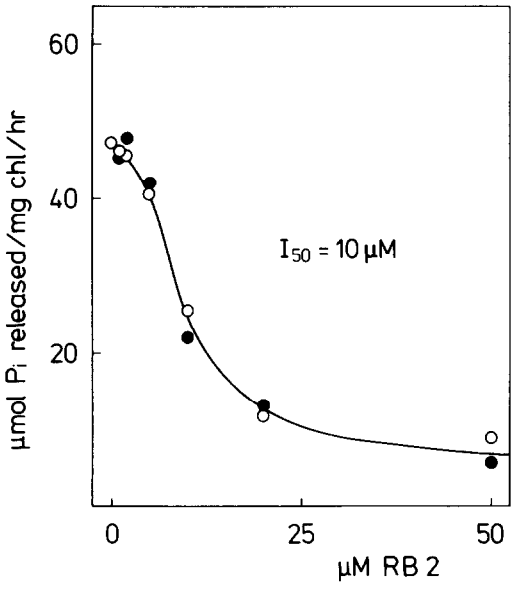


Fig.4. Effect of RB2 on light-triggered ATP hydrolysis by chloroplast RB2 was added either before (open circles) or after the light trigger (closed circles).

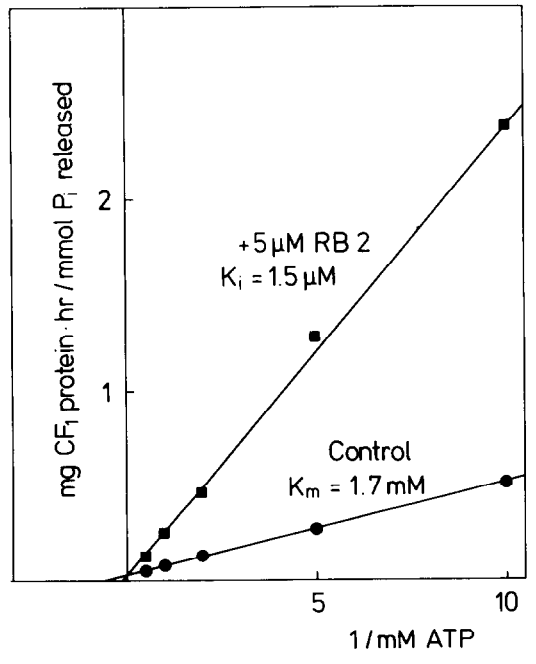


Fig.5. Effect of RB2 on ATP hydrolysis catalyzed by trypsin-activated isolated CF_1 as a function of ATP concentration.

dye (not shown).

In order to localize the inhibition site more precisely, the effect of RB 2 on ATPase activity of isolated CF_1 was investigated. Although CF_1 needs artificial activation by typsin or heat treatment [17], the functional properties of the enzyme (e.g., nucleotide specificity [7]) appear not to be essentially changed compared to its state in situ. Therefore at least qualitatively the effect of RB 2 on solubilized CF_1 should be similar to that on the membrane-bound enzyme. The results show that RB 2 inhibits ATPase activity of CF_1, in this case inhibition is clearly competitive to ATP. The inhibitory effect is even stronger than in the chloroplast reactions. This may be due to a better accessibility of the large inhibitor molecule in the solubilized activated enzyme.

The results suggest that the predominant mode of inhibition is at least formally competitive with the nucleotide molecule on the active site of chloroplast ATPase which is located on the CF_1 part. This is particularly surprising because at the first sight the structural similarity between the substrate and the inhibitor is quite poor. Presumably an additional effect is superimposed in the in situ system which causes the complicated inhibition kinetics in photophosphorylation.

ACKNOWLEDGEMENT

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