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Diethylstilbestrol is a potent inhibitor of the H^+-PPase but not of the H^+-ATPase of *Rhodospirillum rubrum* chromatophores

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In *Rhodospirillum rubrum* chromatophores, diethylstilbestrol inhibits the photoinduced synthesis of ATP and PP_i by the membrane-bound H^+-ATPase and H^+-PPase, respectively. 50% inhibition of ATP synthesis is obtained at 8 μM diethylstilbestrol in the presence of 0.13 μM BChl, while I_{50} for the PP_i formation is 20 μM diethylstilbestrol at the same chromatophore concentration. Diethylstilbestrol also inhibits the hydrolytic activity of the H^+-PPase, both in the membrane-bound and in the solubilized and purified state. Inhibition to 50% is already attained at 3 μM diethylstilbestrol in chromatophores when 1 μM FCCP is present and the BChl-concentration is 0.62 μM. The hydrolysis by the solubilized enzyme has an I_{50} of 5 μM when 5 μg protein/ml is used. In contrast to the PP_i-hydrolysis, the ATPase activity of the chromatophores shows a small activation at low diethylstilbestrol concentration and becomes inhibited at higher concentrations. Also, solubilized F_0F_1-ATPase is activated to a small extent by diethylstilbestrol at the concentrations tested. At low concentrations of BChl, the inhibitory action of diethylstilbestrol on ATP and PP_i synthesis can be reversed by addition of bovine serum albumin. The time dependence and inhibition dependence on the energy state of the membrane and on the BChl concentration are examined for the ATP synthesis. The mechanism of inhibition by diethylstilbestrol is discussed.

Introduction

Rhodospirillum rubrum is a phototrophic bacterium extensively used in bioenergetic studies. It is an organism capable of both photosynthetic and oxidative phosphorylation. It contains two different types of membrane-bound phosphorylating enzyme, the H^+-ATPase [1,2] and the H^+-PPase [3,4]. Both these enzymes are coupled to electron transport by a proton electrochemical gradient.

Chromatophores derived from plasma membranes of light-grown *R. rubrum* have been examined extensively. The chromatophores have the active sites of the two phosphorylating enzymes facing the outer bulk solution, which makes the chromatophores useful when studying phosphorylation, since disappearance and production of substances can easily be monitored. From these chromatophores, the H^+-ATPase [5–7] and the H^+-PPase [8] have been solubilized, characterized and purified.

Coupling of these enzymes to cyclic [9] or non-

Abbreviations: H^+-ATPase, membrane-bound proton translocating ATP synthase; F_0F_1-ATPase, solubilized ATP synthase; H^+-PPase, membrane-bound proton translocating PP_i-synthase; P_i, inorganic phosphate; PP_i, inorganic pyrophosphate; DCCD, *N*,*N*′-dicyclohexylcarbodiimide; BChl, bacteriochlorophyll; MEGA-9, nonanoyl *N*-methylglucamide; FCCP, carbonyl cyanide *p*-trifluoromethoxyhydrazone.

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cyclic [10] electron transport, to each other [11], and to other energy dependent reactions (see Ref. 12 for review) has also been studied from many points of view. In order to scrutinize the enzymes and their interactions with other components and with the energy state of the chromatophores, different inhibitors and uncouplers are important. Energy-transfer inhibitors, such as venturicidin (Strid. Å., unpublished results) and oligomycin [15], which solely inhibit the reactions catalyzed by the ATPase, are often used for this purpose. Using oligomycin when monitoring PP_i synthesis gave an increased rate of PP_i formation [13, 15–17], since the oligomycin decreased dissipation of the proton gradient through less well-coupled H^+-ATPases. Using fluoride ions (F^- being an inhibitor of the H^+-PPase) while studying ATP synthesis [13,16,17] increased the rate of ATP formation by preventing production of PP_i from the phosphate present and thus diminishing dissipation of the protonmotive force through the H^+-PPase.

DCCD, another inhibitor decreasing proton translocation, affects both enzymes when they are bound to the membrane [13,18]. The ATPase can still be inhibited if it is solubilized [19] as the F_0F_1-ATPase, while the PPase as solubilized has lost its sensitivity to DCCD [19].

Diethylstilbestrol has been shown to affect ATP hydrolysis and ATP-dependent proton pumping of plasma ATPases of yeast [20,21], plants [22] and fungi [23,24], of the ATPase of a methanogenic bacterium [25] and of intact mitochondria [26]. Recently, an inhibition of the ATPase activity and the ATP-dependent proton translocation of membrane-bound and purified F_0F_1-ATPase from rat liver mitochondria was reported [27]. This inhibition was shown to be prevented by bovine serum albumin. It was also shown that under certain circumstances, diethylstilbestrol could act as an uncoupler. No report has yet appeared on the effect of diethylstilbestrol on eubacterial systems.

In this paper we present the results of experiments on H^+-ATPase and H^+-PPase (both the membrane-bound and solubilized enzymes) from *R. rubrum* chromatophores. Both synthesis and hydrolysis rates were examined. We also studied the dependence of the action of diethylstilbestrol on its concentration and on the concentration of chromatophores, on time of incubation, on energization of the membrane and on bovine serum albumin concentration. The action of diethylstilbestrol on the electron transport, as studied by reduction and reoxidation of cytochrome *b*, was also examined.

Materials and Methods

Growth of bacteria and preparation of chromatophores

R. rubrum bacteria, strain S_1, were grown anaerobically in light at 30 °C. The medium used was as described in Ref. 28. In the late log phase, after 40 h of growth, the cells were harvested. After washing, chromatophores were prepared according to Ref. 3, except that the cells were broken in a Ribi Cell Fractionator RF-1 and that the chromatophores were washed twice and suspended in 0.2 M glycylglycine (pH 7.4). The concentration of bacteriochlorophyll, BChl, was determined by using the in vivo absorbance coefficient, 140 $mM^{-1} \cdot cm^{-1}$, at 880 nm [29].

Solubilization of F_0F_1-ATPase

F_0F_1-ATPase was prepared from *R. rubrum* chromatophores by solubilization of the membranes, separation from the crude extract by ammonium sulphate precipitation, and further purification by gradient centrifugation. The method used was essentially similar to the one published by Rott et al. [30], except that the detergent nonanoyl *N*-methylglucamide (MEGA-9) [31] was used instead of octyl α-D-glucoside.

Solubilization and purification of membrane-bound H^+-PPase

Solubilization and purification of the H^+-PPase was earlier described in Ref. 32. The method has been slightly modified as follows. The enzyme was solubilized and desalted as in Ref. 32 with the exception that the buffer contained 10 mM $MgCl_2$. 30 ml of the eluate was applied onto a hydroxyapatite chromatography column with a flow rate of 3 ml/min. 30 g of Calbiochem High-Resolution Hydroxyapatite had been rehydrated in the above described buffer and packed onto a 3.2 × 40 cm column. After application of the sample, the col-

umn was washed with 150 ml of the equilibration buffer and then further washed with 150 ml of a buffer identical except in that the $MgCl_2$ content had been raised to 0.2 M. The enzyme was then eluted with a buffer similar to the equilibration buffer, but containing 0.4 M $MgCl_2$. 10-ml fractions were collected. Those fractions showing highest PPase activity (fractions 38–56) were pooled and concentrated to a final volume of 6 ml by ultrafiltration, as in Ref. 32, and kept frozen at −70 °C.

The pH method for measuring of ATP synthesis

A method described by Nishimura et al. [33] for continuous monitoring of ATP synthesis was used. With a pH meter, the scalar protons absorbed during ATP synthesis can be assayed. Depending on the pH of the medium, the stoichiometry of protons consumed per ATP formed varies. At pH 7.6 the stoichiometry is approx. 0.9 [33].

In order to ensure that the chromatophores were dark-adapted before the experiment, the room was kept dark and the cuvette was covered by black fabric. The sample was illuminated from two sides by a LIF-60 55 W halogen-lamp light source (Lorentzen instrument AB, Stockholm, Sweden) through two connected fiberoptical cables. Light was turned on and the normal photophosphorylation activity (100% activity) was recorded for 1 min. Then, still during illumination, diethylstilbestrol (solubilized in 95% ethanol) was added.

Illumination continued for another 1 or 10 min as indicated below. Calibration with 10 mM HCl was performed before and after each experiment.

Control experiments were performed: (i) with chromatophores illuminated without inhibitor addition, (ii) with the assay system itself illuminated, and (iii) with the assay system kept in the dark.

The luciferin / luciferase assay for ATP and PP_i synthesis

ATP synthesis was also followed with the luciferin/luciferase-based bioluminescence method (firefly luciferase, EC 1.13.12.7) [34]. PP_i synthesis was also measured, using this very sensitive method for continuous monitoring of PP_i production [35]. McEnery et al. [27] reported that the inhibition by diethylstilbestrol of ATPase in rat liver mitochondria was prevented by bovine serum albumin. The latter is a constituent of the stock solution of the luciferin/luciferase method, and is used for stabilizing the system. In the experiments performed here, the method and solutions used were according to Ref 35, with the exception that 4 mg/ml poly(vinylpyrrolidone), M_r 360000 was used instead of bovine serum albumin in the stock solution. Poly(vinylpyrrolidone), M_r 360000 stabilized the system even better than bovine serum albumin. 1 mM 1,4-dithioerythritol is used both when assaying synthesis of PP_i and ATP.

Diethylstilbestrol was added either before or during illumination. Photophosphorylation was recorded for at least 2 min. When the effect of addition of bovine serum albumin (to a concentration of 0.1%) was studied, this was added after the diethylstilbestrol had been injected. Control experiments were performed in the presence of chromatophores without addition of diethylstilbestrol. The luminescence output was always calibrated by addition of a known amount of ATP and PP_i, both before and after addition of diethylstilbestrol. A slight lowering of the light emission by the luciferin/luciferase was detected in the presence of diethylstilbestrol and compensated for by the calibrations.

The colorimetric technique for the measuring of hydrolytic activity

The hydrolytic activities were measured as the amount of P_i liberated per min by a modification of the method of Rathbun [36] as described earlier [13]. The PPase assay with chromatophores was performed in presence of 0.55–0.70 μM BChl, and when the solubilized PPase was studied, 10–20 μg of protein was added. In the ATPase experiments 0.66–1.10 μM BChl and 4.7–14.1 μg of protein were present when measuring the activity of chromatophores and solubilized F_0F_1-ATPase, respectively. Incubation with diethylstilbestrol was performed for 10 min before the reaction was started by adding the substrate.

Reduction and reoxidation of cytochrome b

The reduction of cytochrome *b* during illumination and the reoxidation when light was turned off were studied in a double-beam spectro-

photometer as described earlier [37]. The wavelength pair used for cytochrome *b* was 428–450 nm. The assay medium is described in Fig. 5, as are the additions of antimycin, FCCP, diethylstilbestrol and Triton X-100.

Chemicals

Glycylglycine was from Boehringer (Mannheim, F.R.G.) and nonanoyl *N*-methylglucamide from Oxyl (Babingen, F.R.G.). Tris, Triton X-100, 1,4-dithioerythritol, sodium succinate, diethylstilbestrol, adenosine 5′-phosphosulphate, ATP-sulphurylase (ATP : sulphate adenylyltransferase, EC 2.7.7.4), FCCP, antimycin A, bovine serum albumin, $MgAc_2$ and yeast inorganic pyrophosphatase (EC 3.6.1.1) were purchased from Sigma (St. Louis, MO, U.S.A.), and purified firefly luciferase (EC 1.13.12.7), D-luciferin and L-luciferin from LKB Wallac (Turku, Finland). Other chemicals were of reagent grade from commercial sources.

Results

ATP synthesis

The rate of ATP synthesis was studied over a wide range of BChl concentrations. The continuous pH method was used for high chromatophore concentrations, while the luciferin/luciferase technique was used for low concentrations. Figs. 1 and 2 show the rate of ATP synthesis as a function of the concentration of the inhibitor diethylstilbestrol, monitored at different concentrations of chromatophores. The rate 1 min after addition of diethylstilbestrol is given. The three concentrations in Fig. 1 were monitored with the pH method, whereas the lowest amount of BChl (Fig. 2) was studied using the bioluminescence assay. The inhibition is clearly dependent on the BChl concentrations, with half-maximal inhibition at 8, 8, 18 and 40 μM diethylstilbestrol, respectively.

McEnery [27] compared the action of diethylstilbestrol with energy transfer inhibitors as DCCD, oligomycin and venturicidin. The inhibition by oligomycin shows a strong dependence on the incubation time in chromatophores from photophosphorylating bacteria [38,39], whereas the action of venturicidin is practically independent of time after 1 min of incubation [38,39]. Inhibition

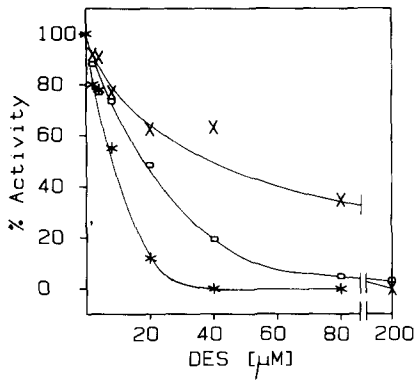

Fig. 1. Rate of ATP synthesis as a function of diethylstilbestrol concentration at different chromatophore concentrations and assayed by the pH method. The experiments were performed using the pH method [33]. The medium consisted of 8.0 mM KH_2PO_4, 12.0 mM $MgCl_2$, 0.4 mM sodium succinate and 100 mM KCl. In (*) 4.5 μM BChl was present, in (○) 18 μM BChl and in (×) 65 μM BChl. Before the addition of diethylstilbestrol, the samples were illuminated for 1 min. The phosphorylation rate obtained was taken as 100% activity. Before and after illumination, the samples were calibrated by additions of 10 μl of 10 mM HCl. The points are the means of at least three experiments. 100 % activity corresponded to between 5 and 7 μmol ATP/μmol BChl per min. DES, diethylstilbestrol.

by DCCD and oligomycin is dependent on the energy state of the membrane in rat liver mitochondria [40], which is also the case for oligomycin in *R. rubrum* chromatophores [39], showing only a small inhibition when incubation was performed in unenergized chromatophores, and much stronger inhibitory action when the chromatophores were illuminated during incubation. At 18 μM of BChl the rate of ATP synthesis was studied as a function of incubation time at several diethylstilbestrol concentrations. During prolonged illumination (10 min) in the absence of diethylstilbestrol, the chromatophores in the controls lost 45% of their phosphorylation activity. If this was compensated for in the experiments with inhibitor added, inhibition reached its maximum within 1 min. The assay system itself was not sensitive to additions of the inhibitor solubilized in 95% ethanol (not shown). We also tested whether the inhibition is energy dependent. Table I shows the results of these studies. It can be seen that the action of diethylstilbestrol is independent of the energy state of the membrane. It is obvious that

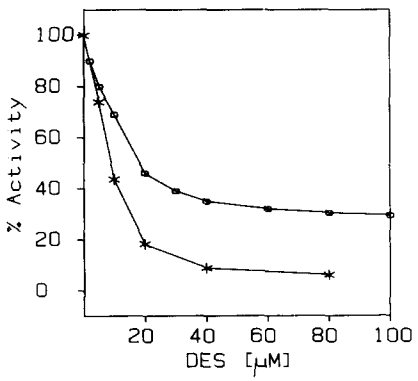

Fig. 2. Rate of ATP and PP_i formation as a function of diethylstilbestrol concentration as assayed with the luciferin/luciferase technique [35]. The medium consisted of 0.1 M glycylglycine (pH 7.75), 1 mM EDTA, 0.13 μM BChl, 10 mM $MgAc_2$, 0.4 mg/ml poly(vinylpyrrolidone) M_r 360000, 1 mM 1,4-dithioerythritol, 0.1 mg D-luciferin, 4 μg L-luciferin, 5 μM adenosine 5′-phosphosulphate, 0.3 U ATP-sulphurylase and purified firefly luciferase to give a response of 1 V to 0.1 μM ATP. The luminometer was calibrated to give a response of 10 mV for the luminometer internal light standard. 1 mM PP_i-free P_i was included in the assay of PP_i formation (○). Before and after diethylstilbestrol addition the experiments were calibrated by addition of known amounts of PP_i. In the studies of ATP synthesis (*) adenosine 5′-phosphosulphate and ATP-sulphurylase were omitted, and 20 μM ADP was included. Here calibration was performed by ATP standard additions. 100% activity corresponded to 0.39 μmol PP_i/μmol BChl per min and 3.5 μmol ATP/μmol BChl per min, respectively. DES, diethylstilbestrol.

TABLE I

RATE OF ATP SYNTHESIS AFTER DIFFERENT INCUBATION TIMES AT ENERGIZATION AND DEENERGIZATION, RESPECTIVELY

20 μM diethylstilbestrol was used in these experiments.

Conditions used	Activity (%)
Control	100
1 min incubation in darkness	45.9 [a]
during illumination	48.4 [b]
10 min incubation in darkness	46.0 [a]
during illumination	45.3 [b]

[a] 100% Corresponds to 5.07 μmol ATP/μmol BChl per min.
[b] 100% Corresponds to 5.76 μmol ATP/μmol BChl per min.

the action of diethylstilbestrol is quite different from that of oligomycin and DCCD [39,40].

The effect of bovine serum albumin on the inhibition by diethylstilbestrol did not give any lasting recovery of phosphorylation when 18 μM BChl and 20 μM diethylstilbestrol was used and the pH method was applied. A slight increase in the synthesis rate was seen the first 0.5 min after the addition of bovine serum albumin, but the rate soon returned to the original rate. This phase might be an effect of insufficient mixing, but increasing the velocity of the magnetic-rod stirrer induced severe noise problems. The bovine serum albumin concentrations ranged from 0.4 μg/ml to 2.6 mg/ml. However, when the luciferin/luciferase technique was used for monitoring ATP synthesis at very low BChl concentrations (0.13 μM), no inhibition by diethylstilbestrol was seen if bovine serum albumin (1 mg/ml) was present in the assay mixture. If instead, bovine serum albumin was added after diethylstilbestrol, the ATP synthesis rate returned to its uninhibited rate. The difference between these results is likely to be a consequence of the different concentrations of BChl in the two experiments, the former being more than 130-fold higher.

PP_i synthesis

PP_i synthesis was also inhibited by diethylstilbestrol with half-maximal inhibition at 18 μM diethylstilbestrol (Fig. 2). In contrast to the inhibition of ATP synthesis, the PP_i formation was not completely inhibited. At a concentration of 100 μM diethylstilbestrol, 30% of the original activity still remained. However, on a molar basis the remaining activities were about the same. As was the case with ATP synthesis, the addition of bovine serum albumin relieved the inhibition by diethylstilbestrol.

ATP hydrolysis

The effect of diethylstilbestrol on the ATP hydrolysis of the *R. rubrum* enzyme appears quite different when compared with the results obtained in rat liver mitochondria [27]. Fig. 3 shows that the ATPase activity of the membrane-bound enzyme in the presence of 1 μM FCCP is slightly activated by diethylstilbestrol up to 20 μM, above which the activity falls again. The shape of this

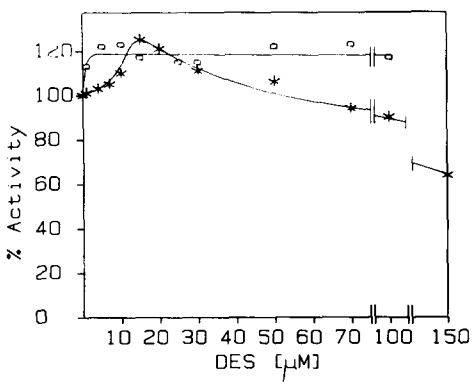

Fig. 3. Rate of ATP hydrolysis in chromatophores and solubilized F_0F_1-ATPase as a function of diethylstilbestrol concentration. The assay medium contained 0.75 mM $MgCl_2$, 0.2 mM sodium succinate, 1 μM FCCP, 1 ml 0.1 M Tris-HCl (pH 7.5) and H_2O to a total volume of 2 ml. In the experiments with chromatophores (*), 0.72 μM BChl was present and when solubilized enzyme (O) was studied, 14.1 μg protein was added. The reaction was started by adding 0.5 mM ATP. 100% activity corresponds to 1.8 μmol ATP/μmol BChl per min or 224.6 μmol ATP/mg protein per min, respectively. DES, diethylstilbestrol.

curve resembles the curve of Fig. 5A of Ref. 23, where the same activity of *Neurospora crassa* mitochondria was tested. Repeating this experiment at higher pH did not give any significant change in the pattern of diethylstilbestrol action on the ATP hydrolysis.

In the absence of FCCP the rate of hydrolysis was increased from 0.45 μmol ATP/μmol BChl per min up to 1.07 μmol ATP/μmol BChl per min when 50 μM diethylstilbestrol was added (not shown). An increase of the diethylstilbestrol concentration up to 100 μM diethylstilbestrol slightly lowered the activity to 0.98 μmol ATP/μmol BChl per min.

The effect of diethylstilbestrol on solubilized F_0F_1-ATPase was an increase of the activity with approx. 20% at 5 μM diethylstilbestrol (Fig. 3). This activation was constant over the whole interval of concentrations tested (up to 100 μM diethylstilbestrol). Half-maximal stimulation was reached at about 1 μM diethylstilbestrol, which is considerably lower than any effect on the other energy-coupled reactions studied (see Table II).

PP_i hydrolysis

An unexpected result, taking into account the minor effects of diethylstilbestrol on membrane-bound ATPase activity and the small activation of the hydrolysis catalyzed by the solubilized F_0F_1-ATPase, is the strong inhibition of the PPase activity, both membrane associated and solubilized (Fig. 4). Addition or omission of FCCP did not have any significant effect. Half-maximal inhibition was seen at 5 μM diethylstilbestrol for the solubilized enzyme and for chromatophores without FCCP present. I_{50} was as low as 3 μM when FCCP was included. 10–20% of the PPase activity still remained in these three different experiments over the diethylstilbestrol concentration range tested.

Reduction and reoxidation of cytochrome b

In order to obtain some information on the mechanism of the inhibitory action of diethylstilbestrol, spectrophotometric studies were performed on cytochrome *b*, which had earlier been

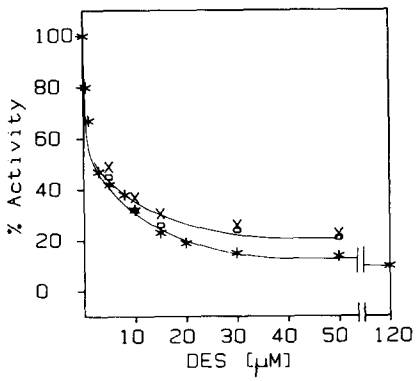

Fig. 4. Rate of PP_i hydrolysis in chromatophores (with and without FCCP added) and solubilized H^+ PPase as a function of diethylstilbestrol concentration. The reation medium consisted of 0.75 mM $MgCl_2$, 0.2 mM sodium succinate; 1 ml 0.1 M Tris-HCl (pH 7.5) and H_2O to a total volume of 2 ml. 0.62 μM BChl was used when chromatophores were studied (* and O) and 10 μg protein when the purified PPase was added (×). FCCP, 1 μM, was present in (*). The reaction was started by addition of 0.5 mM P_i. 100% activity corresponds to 2.4 μmol PP_i/μmol BChl per min in the presence of FCCP and 1.6 μmol PP_i/μmol BChl per min in the absence of FCCP. When the solubilized enzyme was studied, 100% activity corresponded to 74 μmol PP_i/mg protein per min. DES, diethylstilbestrol.

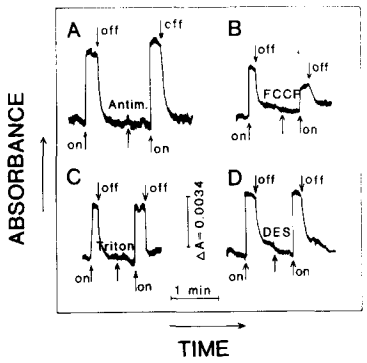

Fig. 5. Typical traces from the studies of cytochrome *b* reduction and reoxidation. The 1.5 ml medium contained 190 mM glycylglycine (pH 7.4), 10 mM $MgCl_2$, 0.33 mM sodium succinate and 57 μM BChl. The wavelength pair 428–450 nm was used to monitor the redox changes of cytochrome *b*. (A) is before and after addition of 1.33 μM antimycin, (B) before and after addition of 1.33 μM FCCP, (C) before and after addition of 0.5% (v/v) Triton X-100 and (D) before and after addition of 80 μM diethylstilbestrol.

shown to respond readily to the addition of both electron transport inhibitors and uncouplers [41]. A comparison between diethylstilbestrol and antimycin, FCCP and the detergent Triton X-100, respectively, clearly showed (Fig. 5) that no significant effect on either the light-induced reduction of cytochrome *b* or the reoxidation in darkness was obtained in the presence of diethylstilbestrol at concentrations where inhibition of ATP and PP_i synthesis occurs.

Discussion

In Table II are summarized the diethylstilbestrol concentrations giving half-maximal inhibition, I_{50}, for the different experiments. Obviously, ATP synthesis can be inhibited up to 50% of its maximal activity by 8 μM diethylstilbestrol when the chromatophore concentration is low (I_{50} is also a function of the BChl concentration, not shown). PP_i synthesis appears to be less sensitive to diethylstilbestrol than the ATP formation, the demand for diethylstilbestrol exhibiting 50% inhibition was at least doubled (18 μM) under identical conditions.

A further look at Table II shows that hydrolysis of PP_i is the most sensitive activity to diethylstilbestrol action tested here. 3 μM diethylstilbestrol was enough to lower the hydrolysis by 50%. The concentration of BChl used in these experiments was 5-fold the concentration giving I_{50} 18 μM diethylstilbestrol for PP_i synthesis as studied by the luciferin/luciferase technique. It is especially interesting to note the inhibition by diethylstilbestrol on solubilized PPase. Although very sensitive to diethylstilbestrol, the PPase activity was never seen to be inhibited to more than 80% within the concentration range tested.

McEnery and Pedersen obtained inhibition of ATPase and ATP-dependent H^+-translocation at low concentrations of diethylstilbestrol in rat liver submitochondrial particles and solubilized F_0F_1-ATPase at pH 7.5 [27]. In contrast, Bowman et al.

TABLE II

I_{50} FOR THE DIFFERENT REACTIONS UNDER VARIOUS CONDITIONS AND THE RATIO BETWEEN THE AMOUNT OF DIETHYLSTILBESTROL ADDED AND AMOUNT OF PROTEIN PRESENT IN THE DIFFERENT EXPERIMENTS

Experiment	Protein (μg)	I_{50} (μM)	Ratio diethylstilbestrol: protein at I_{50} (μmol diethylstilbestrol/mg protein)
pH method	442	8	0.045
	1772	18	0.025
	6560	40	0.015
Luminescence method			
PP_i synthesis	5.2	20	3.8
ATP synthesis	5.2	8	1.5
PP_i hydrolysis			
Chromatophores + FCCP (1 μM)	49	3	0.12
− FCCP	49	5	0.20
solubilized enzyme	10	5	1.0

[23] reported activation of the ATPase activity of *N. crassa* mitochondria at pH 6.7, even at high diethylstilbestrol concentrations (1 mM), whereas at pH 8.2 concentrations above 0.5 mM were inhibitory. In our experiments, at pH 7.5, diethylstilbestrol did not exhibit any substantial inhibitory effect up to a concentration of 0.1 mM on the ATPase of solubilized F_0F_1-ATPase, or of chromatophores (with or without FCCP present) from *R. rubrum*. Raising the pH to 8.5 did not alter the inhibition pattern.

In bovine heart mitochondria, diethylstilbestrol has been proposed to specifically inhibit the F_0 portion of the ATPase complex [27]. It also has been shown to have a more general detergent effect [42], at a concentration of 0.15 μmol diethylstilbestrol per mg protein, releasing a number of proteins from the mitochondrial matrix but not from submitochondrial particles.

The inhibition of the synthesis of ATP and PP_i and of the PPase might be explained by a solubilizing effect. Cardiolipin, a lipid crucial for full hydrolysis activity of the PPase [12], might be removed from the PPase by diethylstilbestrol, and possibly be the reason for the deactivation of the enzyme. A similar inhibitory effect on the PPase in chromatophores has been reported earlier for butanol [14].

The ratios of diethylstilbestrol to protein at I_{50} for our different experiments (using M_r 980 for BChl and 40 mg protein per mg BChl; (Nyrén, P. unpublished observations) when calculating protein content) are also presented in Table II. Clearly, the ratio diethylstilbestrol : protein at 50% inhibition varied considerably between the different methods used, and between the different activities studied.

The solubilizing effect of diethylstilbestrol might have an important influence on the activities of the enzymes; however, it is hardly the only effect, and this is partly demonstrated by the highly varying ratio of diethylstilbestrol to protein giving 50% inhibition, often much lower than the concentration reported previously [42].

Diethylstilbestrol has been shown to inhibit both synthesis and hydrolysis by the membrane-bound H^+-PPase, hydrolysis by the solubilized enzyme and synthesis by the membrane-bound H^+-ATPase of *R. rubrum*. In contrast, the hydrolysis of ATP by both the membrane-bound and the solubilized ATPase is unaffected by the inhibitor. Using 20 μM diethylstilbestrol at low concentrations of chromatophores (or protein) gives maximal inhibition to the affectable activities, while the ATP hydrolysis is unaltered and can be measured accurately without interference.

Acknowledgements

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