EFFECT OF SOME ORGANOMETALLIC COMPOUNDS ON THE PERMEABILITY OF CHLOROPLAST MEMBRANES

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1. Introduction

Previous work in this laboratory has shown that trialkyltin compounds mediate an anion-hydroxide exchange across mitochondrial, erythrocyte and liposome (smectic mesophase) membranes [1, 2]. Kahn [3] has reported that trialkyltin compounds inhibit and uncouple photophosphorylation in isolated chloroplasts. Siegenthaler [4] and Murakami and Packer [5] have reported that when phenylmercuric acetate is added to chloroplasts in a chloride medium, the light-induced light scattering and volume changes resemble those in an acetate medium [6]. Scott et al. [7] have found that organomercury compounds alter the permeability properties of the mitochondrial membrane.

These reports prompted investigation of the effect of organometallic compounds on the permeability properties of the chloroplast membrane, and an extension of our studies on liposomes to the effects of organomercury compounds. Our observations show that tripropyltin chloride and phenylmercuric acetate mediate a chloride-hydroxide exchange across the thylakoid membrane.

2. Materials and methods

Liposomes were prepared as described previously [2]. Chloroplasts were prepared from leaves of pea plants, grown for 14 days in Vermiculite, according to the method of Kalberer, Buchanan and Arnon [8] using 15 g of leaves and 70 ml of medium in an M.S.E. Atomix homogeniser. Chlorophyll was estimated spectrophotometrically by the method of Arnon [9].

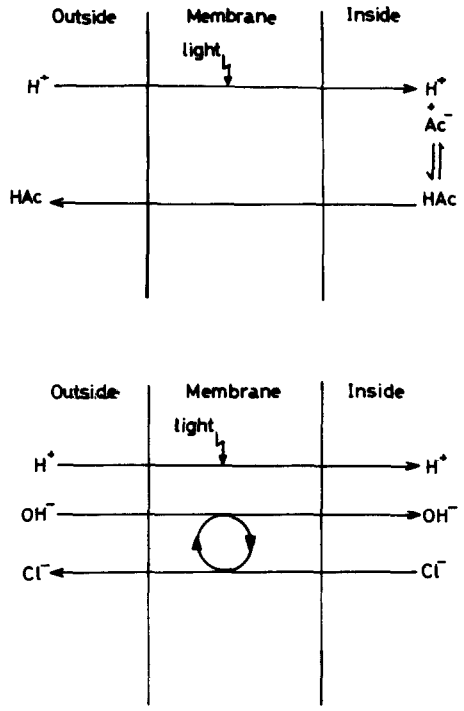
Reagents were obtained as described earlier [2] and in addition, phenylmercuric acetate and sodium iso-ascorbate were from BDH Ltd., PMS (phenazine methosulphate) and *p*-hydroxymercuriphenyl sulphonic acid were from Sigma. All other reagents were of A.R. grade.

Light scattering measurements on liposomes were made as described previously [2]. For light scattering measurements on chloroplasts the signal light was filtered with a Wratten 61 filter and chopped at 250 Hz. The chloroplasts and medium were placed in a 1 cm cuvette with four optical faces and could be stirred with a magnetic follower. Scattered light was detected with a photomultiplier (RCA 931A)protected by a Wratten 61 filter, the signal was fed into an AC amplifier (Tektronix FM 122) which was connected to a potentiometric recorder via a simple rectification circuit incorporating sensitivity and back-off controls. For high angle scattering the photomultiplier was set at 90° to the signal light beam. For low angle scattering the photomultiplier was set at 180° to the signal light which was focussed on to a 5.5 mm stop placed in front of the photomultiplier, which thus received only scattered light deviated by not more than 15°.

Actinic light was provided by a Rank Aldis 2000 projector (150 W quartz iodine lamp), filtered by a Wratten 70 filter and further filtered and focussed on the cuvette by a 1 litre spherical flask filled with water.

3. Results and discussion

Changes in 90° light scattering by chloroplasts sus-



Scheme 1, 2.

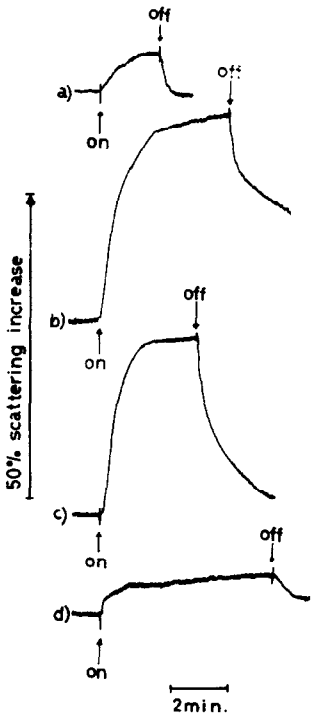


Fig. 1. Effect of organometallic compounds on light-induced changes in 90° light scattering by chloroplasts. Scattering expressed as percentage of basal scattering. Chloroplasts, 5 μg/ml, were suspended in 2.65 ml of 175 mM NaCl containing 20 μM PMS and 10 mM *N*-2-hydroxymethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES) –NaOH buffer pH 8.0, at 20°. Arrows indicate actinic illumination. The suspension was not stirred during the recording. (a) No additions, (b) 0.17 nmole tripropyltin chloride, (c) 10 nmole phenylmercuric acetate, (d) 1.0 μmole *p*-hydroxymercuriphenyl sulphonic acid.

pended in hypotonic media reflect changes not only, in the intra-thylakoid volume but also in shape and possibly membrane conformation. In the range pH 7.5 to 8.0 there is a marked difference between the changes in light scattering (induced by actinic light) of chloroplasts suspended in NaCl media compared to chloroplasts suspended in media containing salts such as sodium acetate. Packer, Deamer and Crofts [6] proposed that in the case of sodium acetate, movement of acetic acid through the membrane is possible, and thus on illumination, when protons move into the intra-thylakoid space, the internal concentration of acetic acid rises and acetic acid diffuses outwards, as shown in Scheme 1. Water moves outwards to restore osmotic balance and the thylakoids shrink. When chloroplasts are suspended in media composed of salts of strong inorganic acids, in the range pH 7.5 to 8 the ions are completely dissociated and do not move in response to the light induced proton gradient; thus there is very little water movement and this is reflected in the small change in light scattering in NaCl medium (fig. 1a).

In the presence of tripropyltin chloride, chloroplasts in NaCl medium respond to actinic illumination with a large change in light scattering (fig. 1b) which is similar to that observed in a sodium acetate medium. It would seem that the trialkyltin-mediated chloride-hydroxide exchange is operative in the thylakoid membrane and that hydroxide ions move into the intra-thylakoid space in response to the light induced pH gradient and cause exit of chloride ions as shown in Scheme 2. The effect of phenylmercuric acetate is very similar (fig. 1c) and it would appear that both the chloride and hydroxide of the phenylmercury radical are lipid soluble and that this compound also mediates a chloride-hydroxide exchange across the membrane. Support for this view comes from the finding that the presence of a strongly dissociated

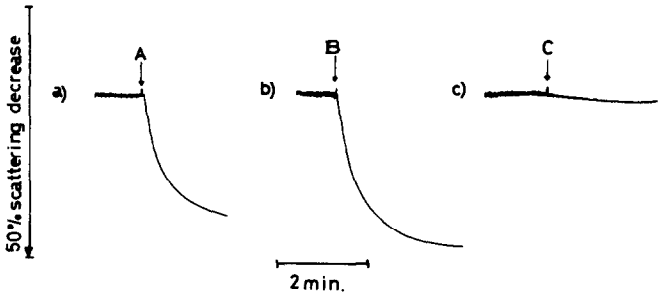


Fig.2. Effect of organometallic compounds on narrow angle light scattering, in the absence of actinic light, of chloroplasts suspended in 175 mM NH_4Cl. Chloroplasts 10 μg/ml, total volume 3.0 ml, pH 7.7, suspension stirred continuously. Arrows indicate addition of A: 5.8 nmole tripropyltin chloride; B: 50 nmole phenylmercuric acetate; C: 50 nmole *p*-hydroxymercuriphenyl sulphonic acid.

group in the molecule, as in *p*-hydroxymercuriphenyl sulphonic acid, greatly reduces its solubility in lipids and abolishes stimulation of the light-induced light scattering change (fig. 1d).

If these proposals for the mode of action of tripropyltin chloride and phenylmercuric acetate are correct, then these compounds should cause swelling, in the absence of actinic light, of chloroplasts in an NH_4Cl medium, by a process similar to the swelling of chloroplasts in solutions of the ammonium salts of weak organic acids [6]. Swelling was followed by recording low angle light scattering, and as shown in fig. 2, addition of tripropyltin or phenylmercuric acetate causes a rapid decrease in scattering but *p*-hydroxymercuriphenyl sulphonate has very little effect.

The use of liposome artificial membrane systems has the advantage that the possibility of modification of existing natural carriers is eliminated. When liposomes containing sucrose medium are suspended in NH_4Cl solution, phenylmercuric acetate induced absorbance decreases, which are similar to, but less extensive than, those induced by tripropyltin (fig. 3a, b). Fig. 3c shows that *p*-hydroxymercuriphenyl sulphate produces only a small initial decrease in absorbance and no continued effect.

In curves (d) and (e) of fig. 3 the liposomes were suspended in a KCl medium. Swelling is limited in extent until both valinomycin and FCCP (carbonylcyanide *p*-trifluoromethoxy phenylhydrazone) are present in addition to phenylmercuric acetate (*p*-hydroxymercuriphenyl sulphonate is inactive in this

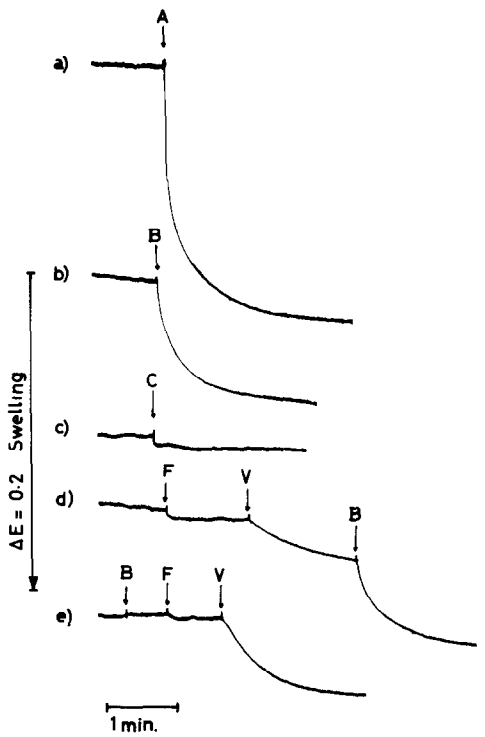


Fig. 3. Effect of organometallic compounds on the swelling of liposomes. 0.1 ml of a 10 mM lecithin liposome preparation in 0.25 M sucrose, 10 mM tris-HCl pH 7.5 was added to (a), (b) and (c) 2.0 ml of 0.2 M NH_4Cl pH 7.3; (d) and (e) 2.0 ml of 0.2 M KCl, 2 mM tris-HCl pH 7.5. The suspension was stirred continuously and arrows indicate the addition of A: 58 nmole tripropyltin chloride; B: 50 nmole phenylmercuric acetate; C: 100 nmole *p*-hydroxymercuriphenyl sulphonic acid; F: 5 nmole FCCP; V: 1.8 nmole valinomycin.

system also). Similar experiments have been reported for the trialkyltin compounds [2] and the interpretation made that the requirement for a proton-conducting uncoupler as well as valinomycin shows that the artificial carrier, in the present case phenylmercuric acetate, mediates either a strictly coupled chloride-hydroxide anti-port or a proton-chloride symport. The latter mechanism is however not compatible with the chemistry of phenylmercuric acetate.

It has been found that the anion-hydroxide exchange mediated by trialkyltin compounds can cause uncoupling of oxidative phosphorylation in mitochondria [10] and it is possible that it may also account for the uncoupling of photophosphorylation by tri-*n*-butyltin chloride observed by Kahn [3] and

the similar exchange mediated by phenylmercuric acetate may account for the uncoupling observed by Siegenthaler [4].

Acknowledgement

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