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The permeability of *Rhodospirillum rubrum* chromatophores to thiocyanate and perchlorate as detected by light-induced fluorochrome fluorescence changes and by photophosphorylation

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SUMMARY

The addition of NaSCN and $NaClO_4$ to *Rhodospirillum rubrum* chromatophores increased the quenching of atebrin fluorescence and decreased the enhancement of 8-anilinonaphthalene-1-sulfonic acid fluorescence without affecting ATP formation. In the presence of NH_4SCN and NH_4ClO_4 the photophosphorylation as well as the light-induced fluorescence changes of both fluorochromes were inhibited. The comparison of these results with the effects of Cl^- and NO_3^- leads to the conclusion that the chromatophore membrane is much more permeable to SCN^- and ClO_4^- than to Cl^- or NO_3^-.

In *Rhodospirillum rubrum* chromatophores the light-induced H^+ uptake[1,2] as well as the light-induced quenching of atebrin fluorescence[3,4] were inhibited by nigericin in the presence of KCl[1,3] or by NH_4Cl[2,4], while ATP formation was not affected. Inhibition of ATP formation in chromatophores by these compounds was obtained only in the presence of valinomycin or nonactin[2,5,6]. In submitochondrial particles a similar requirement for both a valinomycin-type ionophore and nigericin *plus* KCl[7,8] or NH_4Cl[8,9] was reported for uncoupling of energy-conservation reactions. Here too, NH_4Cl by itself inhibited the respiratory-dependent H^+ uptake[10].

A possible explanation of all these results was based on the assumption that the chromatophores and submitochondrial particles, unlike the chloroplasts, are impermeable to Cl^- (refs 6, 9, 10). Therefore, regardless of the nature of the primary

Abbreviations: ANS, 8-anilinonaphthalene-1-sulfonic acid; DAD, 2,3,5,6-tetramethyl-*p*-phenylenediamine; PMS, *N*-methylphenazonium methosulfate.

event in energy coupling, these particles possess an energized state in the presence of Cl^- which is more closely associated with the membrane potential component of the electrochemical H^+ gradient than with the pH gradient. Uncoupling would be obtained only by eliminating both the pH gradient and membrane potential, and the effect of valinomycin should therefore be obtained also by appropriate permeant anions[10, 11]. Indeed, when NH_4Cl was replaced by NH_4NO_3 the respiratory control in submitochondrial particles was abolished even in the absence of valinomycin, suggesting that NO_3^- is a permeant anion[9]. In chromatophores, however, NO_3^- could not replace valinomycin, since NH_4NO_3 like NH_4Cl did not uncouple phosphorylation[2, 3] but inhibited the light-induced quenching of atebrin fluorescence[3, 12].

Recently changes in the fluorescence of atebrin[13], which were shown in chromatophores to be correlated with the pH gradient[3, 4, 12], have also been investigated in submitochondrial particles[14, 15]. Unlike chromatophores, no quenching was observed in the absence of added salts or in the presence of 5 mM KCl, but a permeant anion like NO_3^- induced quenching of atebrin fluorescence by NADH, succinate, ascorbate *plus* *N*-methylphenazonium methosulfate (PMS) or ATP[14, 15]. Moreover, KSCN, NaSCN and $NaClO_4$ were shown to induce a much more pronounced quenching than KNO_3, suggesting that they are more effective permeant anions in these particles[14]. It was therefore of interest to test the permeability of SCN^- and ClO_4^- in chromatophores by comparing the effect of their sodium salts on various light-induced reactions with the effect of their ammonium salts.

The growth of *R. rubrum* cells and the isolation and storage of chromatophores have been described previously[2, 16, 4]. The fluorescence of atebrin and 8-anilinonaphthalene-1-sulfonic acid (ANS) and the simultaneous measurement of photophosphorylation and fluorescence were assayed as outlined by Gromet-Elhanan[3, 4].

2,3,5,6-Tetramethyl-*p*-phenylenediamine (DAD) and the recrystallized magnesium salt of ANS were gifts from Dr A. Trebst, Ruhr University and Dr. E. Daniel, Tel-Aviv University.

As can be seen in Fig. 1 the extent as well as the rate of the light-induced quenching of atebrin fluorescence, which was not affected by the addition of NaCl, was markedly increased by NaSCN. This effect was recorded in the presence of a very low concentration of bacteriochlorophyll (4 μg/3 ml). With higher bacteriochlorophyll concentrations (above 12 μg/3 ml) when the extent of the quenching in the control was already above 80%[4], only stimulation of the rate of quenching could be observed. The ammonium salts of either Cl^- or SCN^- completely eliminated the light-induced quenching of atebrin fluorescence (Fig. 1). ClO_4^- was as effective as SCN^- both in stimulation when added as the sodium salt and in inhibition as the ammonium salt.

The effect of SCN^- and ClO_4^- on the light-induced enhancement of ANS fluorescence is summarized in Table I. At 6 mM both the sodium and ammonium salts of these anions inhibited over 70%, while NaCl even at 60 mM had no significant effect and NH_4Cl rather stimulated the light-induced fluorescence change.

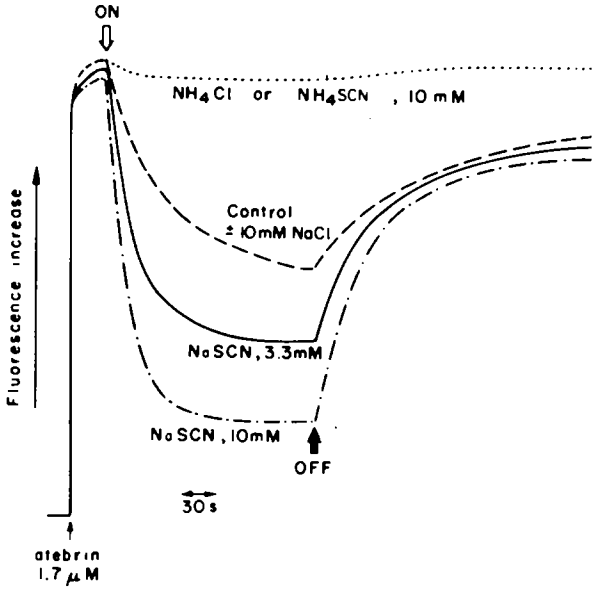

Fig. 1. Effect of the sodium and ammonium salts of Cl^- and SCN^- on the light-induced quenching of atebrin fluorescence. The reaction mixture contained the following components, in a total volume of 3 ml: 30 mM Tricine–NaOH, pH 8.0; 3.3 mM $MgCl_2$; 160 μM succinate; 33 μM PMS and 4 μg bacteriochlorophyll. In some preparations of chromatophores the off rate was very low. It could be stimulated by the addition of 0.01 μM nigericin, which at this concentration and in the absence of K^+ had no effect on the extent or the rate of the light-induced quenching[3].

TABLE I

EFFECT OF THE SODIUM AND AMMONIUM SALTS OF Cl^-, SCN^- AND ClO_4^- ON THE EXTENT OF THE LIGHT-INDUCED STIMULATION OF ANS FLUORESCENCE AND ON PHOTOPHOSPHORYLATION

The reaction mixture contained, in a total volume of 3 ml: 30 mM Tricine–NaOH, pH 8.0; 3.3 mM $MgCl_2$; 0.2 mM DAD; 1.66 mM ADP; 3.33 mM sodium phosphate containing ^{32}P ($5 \cdot 10^6$ cpm), 16.7 μM ANS and 14 μg bacteriochlorophyll. The light-induced extent of ANS fluorescence was recorded in arbitrary units. The control value of ATP formation in the absence of added salts was 353 μmoles/mg bacteriochlorophyll per h.

Salt added	*ANS fluorescence (% of control) with the following salt concentration(mM)*			*ATP formation (% of control) with the following salt concentration(mM)*		
	6	*20*	*60*	*6*	*20*	*60*
NaCl	95	93	80	100	96	100
NaSCN	20	13	0	98	93	80
$NaClO_4$	23	12	0	101	86	70
NH_4Cl	137	126	100	95	81	70
NH_4SCN	30	18	7	78	41	17
NH_4ClO_4	30	22	4	76	41	16

When ATP formation was assayed in the presence of these anions a third type of response was observed (Table I). There was no significant effect of any of the sodium salts or of NH_4Cl at concentrations up to 60 mM. NH_4SCN and NH_4ClO_4 inhibited the phosphorylation but at somewhat higher concentrations than those required to inhibit the ANS fluorescence change (Table I). The same pattern of inhibition of phosphorylation was also obtained when phosphorylation was measured simultaneously with atebrin fluorescence or as the endogeneous, PMS-induced or DAD-induced phosphorylation in the absence of atebrin and ANS.

The ability of NH_4SCN and NH_4ClO_4 to inhibit ATP formation in the absence of valinomycin would indicate, according to Mitchell[11] and Montal *et al.*[10], that the chromatophore membrane is permeable to these anions. If they are indeed permeant anions they should eliminate the membrane potential in the absence of NH_4^+ but not the pH gradient. As a possible test for these properties the effect of NaSCN and $NaClO_4$ on the light-induced changes in the fluorescence of ANS and atebrin have been followed.

In submitochondrial particles an enhancement of ANS fluorescence was induced by ATP, succinate or NADH[17–19]. In these particles changes in ANS fluorescence which mimic the changes induced by electron transport were recently obtained by the generation of a membrane potential with valinomycin in the presence of K^+ (refs 20, 21). In chromatophores a light-induced enhancement in ANS fluorescence[4,12] as well as an ATP- or PP_i-induced enhancement[22] have been reported. The light-induced enhancement was stimulated by NH_4Cl at concentrations which markedly inhibited the H^+ uptake[4,12] suggesting that in the chromatophores as well as in submitochondrial particles this enhancement reflects the membrane potential component of the electrochemical H^+ gradient. The presence of permeant anions which neutralize the membrane potential should therefore decrease this enhancement. Indeed, the addition of NaSCN or $NaClO_4$, but not of NaCl, resulted in a pronounced inhibition of the light-induced enhancement of ANS fluorescence (Table I).

On the other hand, the light-induced quenching of atebrin fluorescence has been shown to reflect the pH gradient in chromatophores[3,4,12] and in subchloroplast particles[23]. It has also been used recently as a method of determining the ΔpH in chloroplasts[24]. The addition of NaSCN or $NaClO_4$, but not NaCl, resulted in a stimulation of the light-induced quenching of atebrin fluorescence (Fig. 1). This stimulation resembles the stimulatory effect of valinomycin in the presence of K^+ on the H^+ uptake in chromatophores[2,5,6], which has also been attributed to the dissipation of the membrane potential[6].

By all the above criteria SCN^- and ClO_4^- indeed behave as permeant anions in the chromatophores: their sodium salts eliminate the membrane potential and stimulate the pH gradient without affecting phosphorylation, while their ammonium salts inhibit all these reactions in the same manner as uncouplers.

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