RELATIONSHIP BETWEEN LIGHT-INDUCED QUENCHING OF ATEBRIN FLUORESCENCE AND ATP FORMATION IN *RHODOSPIRILLUM RUBRUM* CHROMATOPHORES

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1. Introduction

Membrane-bound fluorochromes such as 8-anilino-1-naphthalenesulfonic acid [1] have been employed as probes of the energization of mitochondria and submitochondrial membranes [2]. Energy-dependent uncoupler-inhibited fluorescence changes have been observed [3–6], which could be accounted for by changes in binding of the probe, induced by charge changes of the membrane [6].

Recently a new fluorescent probe, atebrin, which is by itself an uncoupler [7] has been introduced to study the energized state in chloroplasts [8]. A quenching of the atebrin fluorescence was induced by electron transport, ATP hydrolysis or a pH gradient and the quenching decreased when the system was uncoupled. It was therefore concluded that there is a stoichiometric relationship between the generation of energy and the quenching of atebrin fluorescence in chloroplasts.

In the present study the relationship between the quenching of atebrin fluorescence and ATP formation was investigated in *R. rubrum* chromatophores. Evidence will be provided that there is no direct correlation between the two processes, since the light-induced quenchinf of atebrin fluorescence could be completely eliminated under conditions when ATP formation was not affected.

Abbreviations:
HQNO: 2-n-heptyl-4-hydroxyquinoline *N*-oxide;
PMS : *N*-methylphenazonium methosulfate.

2. Experimental

R. Rubrum cells were grown as previously described [9]. The cells were broken and chromatophores were isolated as outlined by Gromet-Elhanan [10]. Bacteriochlorophyll was determined using the extinction coefficient in vivo given by Clayton [11]. The fluorescence of atebrin was measured in an Eppendorf fluorometer which was connected to a Photovolt Model 43 recorder. The excitation light was filtered through a 405–436 nm filter. Fluorescence emission was measured at 470–550 nm through a combination of a C.S. 4-96 Corning filter, a Strand "cinemoid" colour filter No. 62 and 470–3000 nm Eppendorf filter. The samples were illuminated by a 500 W slide projector (without its heat filter) through a Schott RG 645 filter and 3 cm of water.

The reactions were run in cuvettes at room temperature. Unless otherwise stated, the reaction mixture contained the following components in a total volume of 3 ml: Tricine–NaOH buffer (pH 8.0), 20 mM; $MgCl_2$, 3.3 mM; succinate, 160 μM; PMS, 33 μM; HQNO, 1 μM and 12 μg bacteriochlorophyll.

For the simultaneous measurement of photophosphorylation and fluorescence changes the reaction mixture contained in addition 1.66 mM ADP and 3.33 mM sodium phosphate containing 5×10^6 cpm ^{32}P. To each experiment 1.67 μM atebrin was added and the fluorescence was recorded. The cuvette was then illuminated for 3 min, with a continuous recording of the quenching of fluorescence. After an additional 2 min in the dark when the dark decay rate and extent were recorded, perchloric acid was

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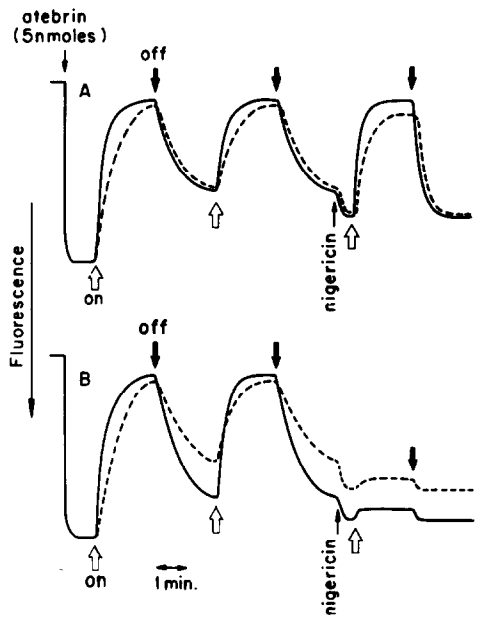

Fig. 1. The light-induced quenching of atebrin fluorescence and the effect of phosphorylating reagents, KCl and nigericin on it. In A, the reaction mixture was as described under Experimental; B contained in addition 130 mM KCl. The broken lines represent experiments carried out in the presence of 3.33 mM sodium phosphate and 1.66 mM ADP. Where indicated 0.1 μM nigericin was added.

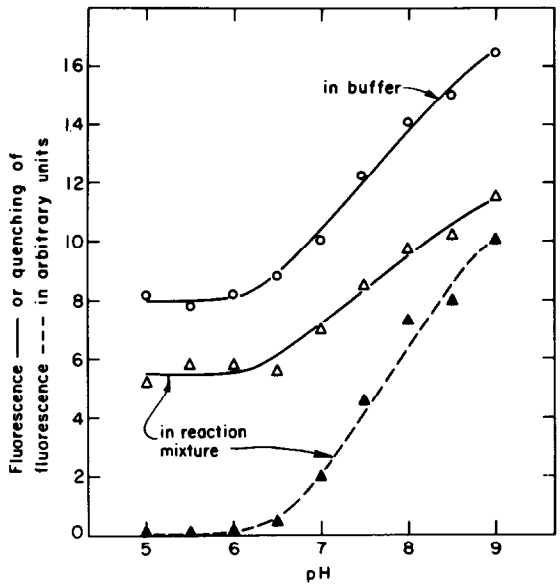

Fig. 2. The effect of pH on the atebrin fluorescence and on the light-induced quenching of fluorescence. The fluorescence was followed in buffer only and in the complete reaction mixture (see Experimental). In the latter system both the fluorescence and the light-induced quenching of fluorescence were measured in the same cuvette. 20 mM Tricine buffer was used between pH 9.0 and 6.5 and 20 mM 2-(*N*-morpholino) ethanesulfonic acid (MES) buffer was used between pH 7.0 and 5.0. The values obtained at pH 7.0 and 6.5 were the same whether tricine or MES buffer were used.

Table 1

Effect of ammonium nitrate and nigericin on the light-induced quenching of atebrin fluorescence and on photophosphorylation. The reaction mixture was as described under Experimental. Where indicated 130 mM KCl was added.

Addition	Fluorescence quenching (% of total fluorescence)[a]		ATP formation (% of control)[b]	
	−KCl	+KCl	−KCl	+KCl
None	89	92	101	93
NH_4NO_3, 2mM	40	45	96	85
NH_4NO_3, 6 mM	19	17	90	75
Nigericin, 0.03 μM	76	33	104	77
Nigericin, 0.1 μM	68	14	108	70
Nigericin, 0.3 μM	44	0	90	60
Nigericin, 1.0 μM	17	0	88	48

[a] The total fluorescence varied within less than 10% in the presence of the added compounds.

[b] The control values of ATP formation in the absence of atebrin were 587 and 543 μmoles/mg bacteriochlorophyll/hr without and with KCl respectively.

added to 3% (w/v) and ATP formation was assayed according to the method of Avron [12].

3. Results and discussion

In agreement with the observation of Kraayenhof in chloroplasts [8] illumination in the presence of chromatophores was also found to induce a quenching of the fluorescence of atebrin (fig. 1A, B). In the chromatophores as in chloroplasts the quenching was observed at atebrin concentrations far below those required for inhibition of ATP formation [13]. However, unlike in chloroplasts [8], the quenching in chromatophores decreased from 90% to 28% of the total dark fluorescence when the atebrin concentration was raised from 1.6 to 10 μM although ATP formation was inhibited by less than 20% even at 10 μM atebrin. The light induced quenching was not significantly affected by the presence of the phosphorylating reagents or of KCl (fig. 1 A, B). It was, however, completely inhibited by nigericin in the presence of KCl (fig. 1B) but not in its absence (fig. 1A).

Nigericin was reported [14] to inhibit the pH effect without affecting ATP formation in chromatophores in the presence of KCl. Ammonium salts were also shown to affect the H^+ uptake and ATP formation in a way similar to nigericin but independent of KCl [9]. The effect of nigericin and ammonium nitrate on the fluorescence quenching and on ATP formation was therefore measured simultaneously (table 1). ATP formation was found to be relatively resistant to the combined effect of nigericin and atebrin or ammonium nitrate and atebrin under conditions which completely eliminated the light-induced quenching. The above results indicate that the quenching might reflect changes in pH rather than the energized state leading to ATP formation. The quenching was indeed found to exhibit a pronounced pH dependence (fig. 2). It was 80% inhibited at pH 7.0 and completely disappeared below pH 6.5, whereas the fluorescence signal in the absence or presence of chromatophores in the dark decreased at pH 6.5 to 50% of its value at pH 9.0 and no further decrease was observed on lowering the pH to 5.0. This steep pH dependence of the light-induced quenching of atebrin fluorescence is in contrast with the observed fluorescence changes of 8-anilino-1-naphthalenesulfonic acid in mitochondria which were reported to be insensitive to alterations of pH [2].

It is therefore concluded that at least in chromatophores the light-induced quenching of atebrin fluorescence can not be used as a probe for the energized state. The possibility that the quenching reflects changes in binding of the atebrin by the chromatophores, which are dependent on the light-induced differences in pH between the outside and the inside (and are therefore inhibited by ammonium nitrate or by nigericin and KCl), is under investigation.

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References

[1] G. Weber and D.J.R. Laurence, Biochem. J. 56 (1954) 31P.
[2] A. Azzi, B. Chance, G.K. Radda and C.P. Lee, Proc. Natl. Acad. Sci. U.S. 62 (1969) 612.
[3] L. Packer, M.P. Donovan and J.M. Wrigglesworth, Biochem. Biophys. Res. Commun. 35 (1969) 832.
[4] B. Chance, A.Azzi, I. Mela, G. Radda and H. Vainio, FEBS Letters 3 (1969) 10.
[5] B. Chance and P.C. Lee, FEBS Letters 4 (1969) 181.
[6] A. Azzi, Biochem. Biophys. Res. Commun. 37 (1969) 254.
[7] M. Avron and N. Shavit, Biochim. Biophys. Acta 109 (1965) 317.
[8] R. Kraayenhof, FEBS Letters 6 (1970) 161.
[9] S. Briller and Z. Gromet-Elhanan, Biochim. Biophys. Acta 205 (1970) 263.
[10] Z. Gromet-Elhanan, Biochim. Biophys. Acta 223 (1970) 174.
[11] R.K. Clayton, in: Bacterial Photosynthesis, eds. H. Gest, A. San Pietro and L.P. Vernon (Antioch Press, Yellow Springs, Ohio, 1963) p. 495.
[12] M. Avron, Biochim. Biophys. Acta 40 (1960) 257.
[13] H. Baltscheffsky, Biochim. Biophys. Acta 40 (1960) 1.
[14] N. Shavit, A. Thore, D.L. Keister and A. San Pietro, Proc. Natl. Acad. Sci. U.S. 59 (1968) 917.