ENERGY-LINKED CHANGES OF THE MEMBRANE OF *RHODOSPIRILLUM RUBRUM* CHROMATOPHORES DETECTED BY THE FLUORESCENT PROBE 8-ANILINONAPHTHALENE-1-SULFONIC ACID

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1. Introduction

ANS, 8-anilinonaphthalene-1-sulfonic acid, fluorescence changes have been shown to measure structural transitions of proteins [1–4]. In the mitochondrial membrane ANS fluorescence changes can be induced by ATP, succinate or NADH [5–7]. In the lobster axon, they are parallel to the action potential [8], and in the membrane of microsomes [9, 10] they can be induced by ions. ANS fluorescence changes in the fragments of the electric organ of Electrophorus [11] have also been described.

Changes in the ANS binding upon attainment of the energized state have been measured in mitochondria and submitochondrial particles and have been attributed to a charge change of the mitochondrial membrane [12, 13]. Intrinsic changes of membrane hydrophobicity have been considered also to occur in the energized state of the mitochondrial membrane [14].

The energized state in *Rhodospirillum rubrum* chromatophores can be obtained by light, ATP or PPi [15, 16]. Spectral changes of endogenous carotenoids in chromatophores, obtained in the dark by addition of PPi or ATP, have been found to be associated with energy conservation [17]. The rapid kinetics of this carotenoid band shift has been shown to be concomitant with redox changes of cytochromes [17].

In the present study, an interaction of ANS with membranes of *Rhodospirillum rubrum* chromatophores has been discovered and investigated, together with an effect of ATP and PPi on the dye fluorescence.

2. Materials and methods

Fluorescence changes of ANS were measured in an Eppendorf fluorometer. Excitation light was filtered through a 366 nm interference filter, emission light through a Wratten 2E gelatin filter. Fluorescence changes were recorded on a Hitachi Perkin Elmer potentiometric recorder. *Rhodospirillum rubrum* chromatophores were grown and prepared as previously described [18]. ANS (from K and K) was recrystallized twice from hot solutions of its Mg salt. All other reagents were analytical grade.

3. Results

3.1. *Pyrophosphate and ATP induced ANS changes in the chromatophore membrane*

The addition of PPi or ATP to the membrane of

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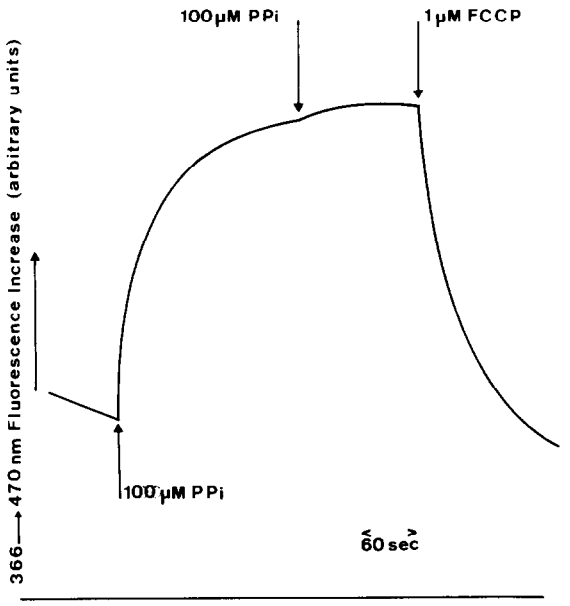

Fig. 1. Pyrophosphate induced ANS response in *Rhodospirillum rubrum* chromatophores. Experimental conditions: *Rhodospirillum rubrum* chromatophores (0.11 μM Bacteriochlorophyll) were suspended in 0.2 M glycyl-glycine pH 7.4, 5 mM $MgSO_4$ and 53 μM ANS.

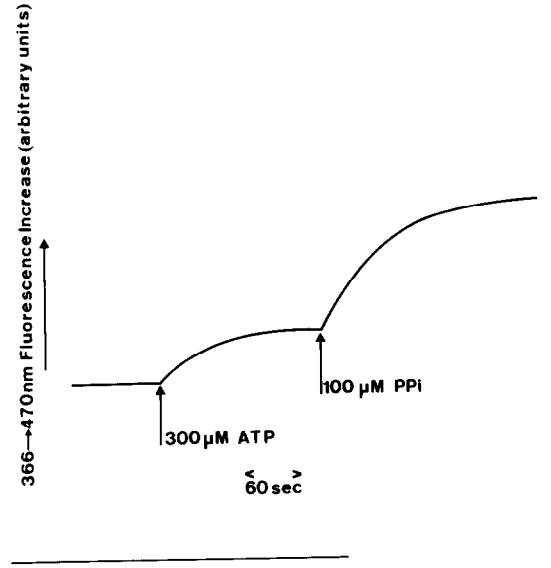

Fig. 2. ATP and pyrophosphate induced ANS response in *Rhodospirillum rubrum* chromatophores. Experimental conditions as in fig. 1.

chromatophores has been shown to result, under the appropriate conditions, in a change in absorbance of carotenoids [12] in a reversal of electron transport [13], and in bromthymol blue absorbance changes [16]. All these effects have been interpreted as a change in the energy state of the membrane.

In fig. 1 the PPi induced response of the ANS fluorescence in chromatophores is reported. The addition of 100 μM PPi to a suspension containing 0.11 μM chlorophyll, 53 μM ANS and 5 mM $MgSO_4$ induces a 20% increase in the ANS fluorescence. One of the characteristics of this record is the half-time of the ANS response of about 30 sec. A second addition of PPi does not result in any significant further fluorescence change. 1 μM FCCP, after the two additions of PPi induced a reversal of the ANS fluorescence change with a half-time similar to that of the PPi induced change. An effect similar to that shown by FCCP in fig. 1 was exhibited by valinomycin in the presence of K^+ ions.

In the experiment of fig. 2 the effect of ATP on the fluorescence of ANS in chromatophores is reported. 300 μM ATP induced an ANS fluorescence increase.

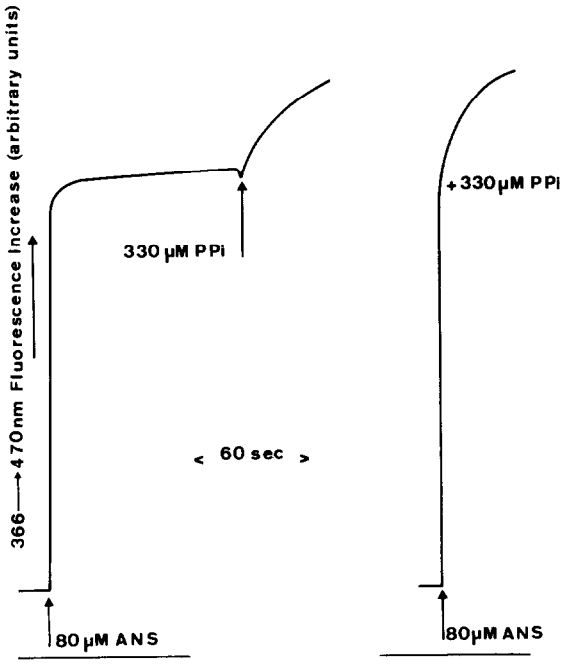

Fig. 3. ANS interaction with *Rhodospirillum rubrum* chromatophores in the presence and absence of pyrophosphate. Experimental conditions as in fig. 1. ANS was added at the concentration indicated in the figure.

100 μM PPi added subsequently produced a further fluorescence increase, twice as large as the ATP induced change. Half-times of ATP and PPi induced changes appear to be the same.

3.2. *ANS interaction with the membrane of chromatophores in the non-energized and in the energized state*

The addition of ANS (50 μM) to a suspension of chromatophore membranes (fig. 3) in the absence of any added pyrophosphate, results in a rapid ANS fluorescence increase (limited by the response time of the recorder of about half a second) which is consistent with the binding of ANS in a hydrophobic environment on the membrane. A smaller and slower fluorescence increase is also associated with binding of ANS to the membrane of chromatophores in the non-energized state. Addition of PPi (300 μM) induces the expected fluorescence increase. If PPi is added to the chromatophore membranes in the absence of ANS and about 4 min are allowed to the membrane to become fully energized, on the addition of ANS the rapid fluorescence increase appears to be unaltered, while the slow component appears to be increased several times and has kinetics and extent similar to the PPi induced change in the presence of ANS.

4. Discussion

Interaction of ANS with the membrane of *Rhodospirillum rubrum* chromatophores, results in a fluorescence increase of the dye indicating that ANS binding sites on the membrane have a character more hydrophobic than water. The fluorescence increase induced by PPi, ATP and succinate is related to the property of these substances of energizing the membrane, and is reversed or inhibited by agents, such as uncouplers (and oligomycin in the case of ATP) which interfere with energy conservation. These results appear to be quite similar to what is observed in submitochondrial particles, where ANS exhibits a fluorescence increase associated with membrane energization [5, 13].

An energy linked ANS fluorescence change can be interpreted in different ways. First, due to the fluorescent properties of the dye, sensitive to the polarity of its environment [18, 19], the energy linked ANS fluorescence increase can be the result of an increase in membrane hydrophobicity.

A second possible interpretation is that energy conservation can be associated with an increased binding of ANS to chromatophores membranes. This interpretation would be consistent with similar binding changes of ANS in mitochondrial fragments associated with energy conservation [12, 13]. It would also be consistent with the binding changes of organic anions (ANS is anionic) observed by Skulachev and his co-workers in both mitochondrial fragments and *Rhodospirillum rubrum* chromatophores [20, 21].

A more direct approach to the problem of the mechanism of ANS fluorescence change in *Rhodospirillum rubrum* chromatophores is afforded by the experiment of fig. 3. In this experiment it appears that the ANS fluorescence increase induced by PPi has approximately the same kinetic characteristics of the interaction of the dye with previously energized membranes, as far as the slow component is concerned.

This experiment appears to indicate that addition of PPi produces an increase in ANS binding to the membrane and that the time course of the fluorescence change reflects more the kinetics of the dye binding to the membrane than the kinetics of the onset of the energized state.

It appears that the early events indicated by the rapid carotenoid band shift [17] can produce a membrane change followed by ANS binding changes. It is, however, also possible that a primary event of energy conservation can lead directly to ANS binding changes. The rate limiting event of the kinetics of ANS fluorescence changes obtained by ATP and PPi would appear to be the changes in binding of this fluorescent probe to its appropriate sites on the chromatophore membrane.

References

[1] L. Stryer, J. Mol. Biol. 13 (1965) 482.
[2] G. Weber and D.J.R. Lawrence, Biochem. J. 56 (1954) 31.
[3] W.O. McClure and G.M. Edelman, Biochemistry 6 (1967) 559.
[4] G. Weber, Advan. Protein Chem. 8 (1953) 415.
[5] A. Azzi, B. Chance, G.K. Radda and C.P. Lee, Proc. Natl. Acad. Sci. U.S. 62 (1969) 612.

[6] A. Datta and H.S. Penevfsky, J. Biol. Chem. 245 (1970) 1537.
[7] K. Nordenbrand and L. Ernster, Europ. J. Biochem. 18 (1971) 258.
[8] I. Tasaky, A. Watanabe, R. Sandlin and L. Carnay, Proc. Natl. Acad. Sci. U.S. 61 (1968) 883.
[9] B. Rubalcava, D. Martinez de Munoz and C. Gitler, Biochemistry 8 (1969) 2742.
[10] B. Gompaert, F. Lantelme and R. Stock, J. Membrane Biol. 3 (1970) 241.
[11] M. Kasai, J.P. Changeux and L. Monneric, Biochem. Biophys. Res. Commun. 36 (1969) 420.
[12] A. Azzi, Biochem. Biophys. Res. Commun. 37 (1969) 254.
[13] A. Azzi, P.L. Gherardini and M. Santato, J. Biol. Chem. 246 (1971).
[14] G.H. Dodd and G.K. Radda, Biochem. J. 114 (1969) 407.
[15] A.W. Frenkel, J. Am. Chem. Soc. 76 (1954) 5568.
[16] M. Baltscheffsky, Nature 216 (1967) 241.
[17] M. Baltscheffsky, Arch. Biochem. Biophys. 130 (1969) 646.
[18] M. Baltscheffsky, Arch. Biochem. Biophys. 133 (1969) 46.
[19] D.C. Turner and L. Brand, Biochemistry 7 (1968) 3381.
[20] L.L. Grinius, A.A. Jasaitis, Yu.P. Kadziauskas, E.D. Liberman, V.P. Skulachev, V.P. Topali, L.M. Tsofina and M.A. Vladimirova, Biochim. Biophys. Acta 216 (1970) 1.
[21] P.L. Isaev, E.A. Liberman, V.D. Samuilov, V.P. Skulachev and L.M. Tsofina, Biochim. Biophys. Acta 216 (1970) 22.