THE INTERACTION OF 1-ANILINO-8-NAPHTHALENE SULPHONATE WITH ERYTHROCYTE MEMBRANES

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1. Introduction

The fluorescence of 1-anilino-8-naphthalene sulphonate (ANS) is highly sensitive to the microenvironment of the chromophore [1]. Because of this property ANS has been used as a probe for hydrophobic protein binding sites [1,2], for protein conformational states [3], for following rates of allosteric transitions [4], and for reading out the dymamic state of membranes in submitochondrial particles [5].

The present communication reports part of our programme for elucidating the nature of the interaction of ANS with membranes. Since erythrocyte membranes have been widely studied with respect to structure and function they were used in this work.

2. Materials and methods

Human erythrocyte stroma was prepared by the method of Dodge et al. [6]. Lipid extracted protein was prepared by the method of Rega et al. [7]. Protein concentrations in the stroma and extracted material were determined by the method of Lowry et al. [8] using crystalline BSA as standard. ANS was purified as described before [9]. All other reagents were obtained from commercial sources and were of the highest available purity. Fluorescence measurements were made on a Zeiss spectrofluorimeter or on a more sensitive instrument developed in this laboratory [10].

3. Results

ANS interacts with erythrocyte membranes and with membrane protein. This is accompanied by a blue shift in the emission of ANS of 70 mμ and an enhancement of about 100-fold (fig. 1). The latter value is derived from double reciprocal plots of fluorescence against membrane concentration by extrapolation to infinite binding. The rate of this interaction is biphasic with the intact stroma, but only the fast phase is observed with sonicated membrane or with membrane protein (fig. 2). The dissociation

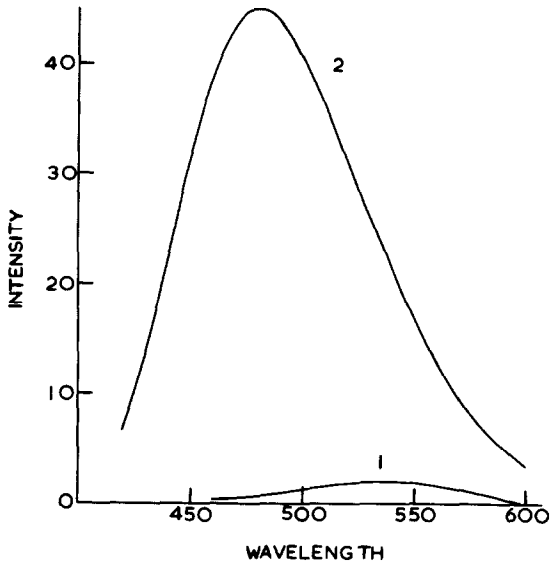

Fig. 1. Fluorescence emission of ANS (20 μM) in buffer and in the presence of stroma. 1. ANS in isotonic Na-phosphate buffer, pH 7.4. 2. ANS with stroma (0.5 mg/ml protein) in pH 7.4 buffer.

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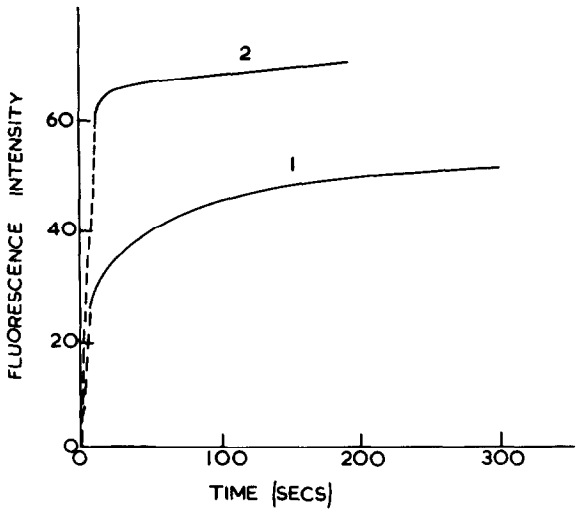

Fig. 2. Rate of interaction of ANS (20 μM) with stroma (0.07 mg/ml in protein). 1. Intact stroma 2. Sonicated membrane.

constants, the numbers of "sites", the fluorescence enhancement values and polarizations are summarized in table 1.

The interaction is strongly pH-dependent with a pK_a of 3.2–3.5 (fig. 3). ANS fluorescence in water, 50% aqueous ethanol (v/v) and bound to BSA is independent of pH in the range of 1.0–9.0.

The fluorescence is also sensitive to changes in ionic strength brought about by adding NaCl. The variation with NaCl concentration is linear between an ionic strength of 0.15–2.5 and the fluorescence increase is 3-fold over this range.

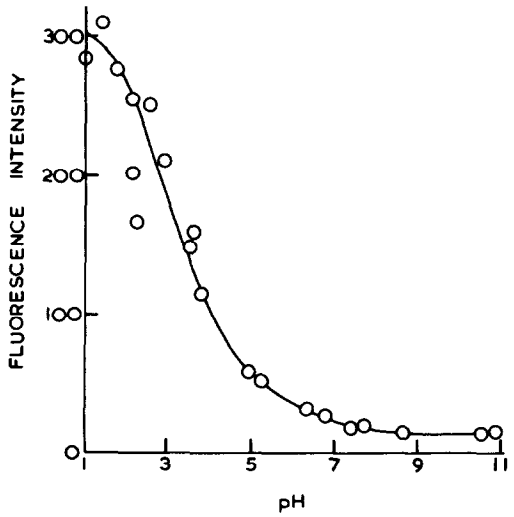

Fig. 3. pH dependence of ANS (20 μM)/stroma (0.07 mg/ml in protein) fluorescence in isotonic buffer.

Table 1
Interaction of ANS with various membrane preparations.

Sample	Enhancement of fluorescence	No. of ANS sites (μmoles/g protein)	K_{diss} (μM)	Polarization
Intact stroma	170	23	41	0.21
Stroma pH 3.1	740	44	9	0.23
Sonicated stroma	23	82	10	0.24
Membrane protein	27	89	19	0.20
Membrane protein pH 3.0	800	–	–	–
Stroma in 2.7 M NaCl, pH 6.5	–	–	–	0.23

Samples are in isotonic Na-phosphate buffer, pH 7.4, or in isotonic NaCl adjusted to the pH stated. Excitation is at 380 mμ, emission at 480 mμ.

4. Discussion

The shift and enhancement in ANS fluorescence suggest that the dye is transferred to a less polar environment than water. The fast and slow rates of enhancement probably correspond to interactions at the outside and diffusion into the membrane. The lack of a slow phase in sonicated fragments and membrane protein supports this.

The pH-dependence implicates sialic acid residues which when ionized interact unfavourably with the negatively charged dye. NaCl is known to bring about shrinkage of the membrane and the enhancement of fluorescence at high ionic strength is consistent with exclusion of some water molecules from the microenvironment of the dye.

The polarization of fluorescence for ANS in the membrane is considerably lower than that for other ANS protein interactions [2,9] but is similar to that observed for submitochondrial particles [5]. This suggests that ANS is in a relatively mobile phase. This together with the binding data, involving a variable number of "sites", and the observation that ANS fluorescence is also enhanced by phospholipid micelles in water [11] suggests that the interaction is not a true binding but constitutes a phase problem. The numbers of "sites" are a measure of the solubility of the dye in the nonpolar phase of the membrane in which structural rearrangements are reflected by the probe.

Acknowledgement

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