Fluorescence of Human Erythrocyte 'Ghosts' on Treatment with 8-Anilino-1-Naphthalene Sulfonate

EDELMAN and MCCLURE[1] and STRYER[2] have recently reviewed the use of 8-anilino-1-Naphthalene Sulfonate (ANS) and similar compounds as fluorescent probes for the hydrophobic regions of protein molecules. We have found that a suspension of ghosts fluoresces strongly in a solution of ANS at pH 7.4. Most of this work had been completed when FREEDMAN and RADDA[3] reported the interaction of ANS with ghosts.

Materials and methods. Hemoglobin-free ghosts were prepared by hypotonic hemolysis at pH 7.4[4,5]. The number of ghosts in a stock suspension was determined in a Coulter Counter, model B. The Mg salt of ANS (Turner, Palo Alto, Calif.) was dissolved in 0.13 *M* phosphate, pH 7.4. Fluorescence intensity was measured in an Aminco-Bowman spectrofluorometer. Later measurements were made with a Photovolt model 54 Fluorescence Meter with primary filter No. 5464 and secondary filter No. B470. Both instruments gave similar results.

Results. Addition of ghosts to a 0.20 m*M* solution of ANS gave marked enhancement of fluorescence, with maximum wavelength for activation at 390–400 nm and for emission at 465–475 nm. As increasing volumes of ghost suspension (5.2×10^9 cells, 3.0 mg of protein, per ml) were added to 5 ml of 0.12 m*M* ANS in buffer, the fluorescence intensity increased proportionately up to 0.20 ml of ghosts added. Addition of 1% bovine serum albumin (BSA) to 5 ml of ANS in buffer gave a linear response up to 0.10 ml added. Thus, on a protein weight basis, BSA yielded 3.2 times the fluorescence intensity compared with ghosts. Addition of 1 m*M* $CaCl_2$ to an ANS-ghost system increased the fluorescence by 8%. Addition of 1 m*M* ATP or 0.1 m*M* Mersalyl or ouabain had no effect.

Triton X-100 (a mixture of polyoxyethylene ethers) at 0.05% concentration 'solubilizes' ghosts and releases certain enzyme activities[6]. However, the fluorescence of this ghost solution could not be examined, as solutions of Triton X-100 themselves fluoresce strongly. WEBER and LAURENCE[7] first reported the enhancement of ANS fluorescence by organic solutes.

In order to estimate the binding of ANS to ghosts, 0.10–1.00 ml aliquots of the stock ghost suspension were added to 5.0 ml of 0.12 m*M* ANS in buffer. After 5 min the ghosts were centrifuged down in a high-speed centrifuge and the concentration of unbound ANS was determined from the absorbance of the supernatant at 350 nm, after correction for the appropriate blanks. The percentages of ANS bound, for the varying volumes of ghosts, were: 0.100 ml, 5%; 0.200 ml, 10%; 1.00 ml, 42%. It was estimated that at the lower ghost concentrations one cell bound 3×10^7 molecules of ANS. On a protein weight basis, 1 mg of ghost protein bound 1.0×10^{-7} moles of ANS. WEBER and YOUNG[8] found that 1 mole of BSA bound a maximum of 5 moles of ANS; this equals 7.6×10^{-8} moles/mg of protein.

As measured from a molecular model, the area of the ANS molecule is about 120 Å^2. The surface area of the human erythrocyte is about 160 μ^2. If it be assumed that a ghost has the same area (although the volume is somewhat smaller), then 3×10^7 molecules of ANS would cover 43 μ^2, or about $^1/_4$ of the cell surface, if bound flat on the surface.

When an ANS-ghost preparation was examined in a fluorescence microscope, the fluorescent membranes could be readily seen, and their shapes clearly delineated. The definition is, however, somewhat inferior to that obtained with phase-contrast optics[9,10].

Zusammenfassung. Erythrozytenmembranen fluoreszieren in einer Lösung von 8-Anilino-1-Naphthalin Sulfonat bei Phosphatpuffer pH 7.4. Während Kalziumionen die Fluoreszenz leicht verstärken, bleiben ATP, Mersalyl und Ouabain ohne Wirkung. Klare Membranbeobachtung mit dem Fluoreszenzmikroskop.

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Fluorescence of erythrocyte ghosts and of bovine serum albumin in a 0.12 m*M* solution of ANS in buffer pH 7.4

ml of ghost suspension	*I*	1% bovine serum albumin (ml)	*I*
0.050	279	0.005	275
0.100	565	0.010	515
0.150	823	0.020	1050
0.200	1100	0.030	1700
0.250	1220	0.040	2200
0.300	1390	0.050	2900
		0.100	2900

Fluorescence intensity. *I*, is the product of the meter reading and the photometer multiplication factor.

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[9] Addendum. The paper by B. RUBALCAVA et al. (Biochemistry *8*, 2742 (1969)) appeared after this report had been submitted for publication.
[10] This work was supported by the Medical Research Council of Canada.

DNS-Synthese, Blastentransformation und Mitosen in gemischten, homologen Granulozyten-Lymphozytenkulturen

Die Transformation von menschlichen Blutlymphozyten in vitro in der Gegenwart homologer Leukozyten[1] wird als Konsequenz des Histokompatibilitätsunterschiedes der Zellpopulationen interpretiert, indem immunkompetente Lymphozyten nichtidentische Transplantationsantigene der fremden Zellmembran erkennen und dies unter anderem mit Blastentransformation, Mitose und gesteigerter Nukleinsäuresynthese beantworten[2–4].