INTERACTION OF FLUORESCENT PROBES WITH PLASMA MEMBRANES FROM RAT LIVER AND MORRIS HEPATOMA 3924A

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1. Introduction

The physico-chemical aspects of plasma membranes are responsible for a number of cell properties, such as adhesiveness, contact inhibition of growth and movement, electrical coupling, cell fusion etc. [1].

The technique of fluorescent labeling [2–4] has been applied mostly to the study of the red blood cell [5,6], but little is known about plasma membranes such as that of the liver cell.

The possible information afforded by such a technique regard the polarity, viscosity, distances and interactions of the sites of probe binding [4]. Moreover, fluorescent probes may be used for comparing physicochemical characteristics of different membranes.

In this study the fluorescent probe 1-anilinonaphthalene-8-sulfonate (ANS)has been employed in liver and Morris hepatoma plasma membranes in an attempt to explore some of their physical characteristics; such as ion binding properties, polarity and protein–lipid interactions.

2. Materials and methods

Adult male albino rats were used for the isolation of liver plasma membranes. Morris hepatoma 3924A [7] was grown in both hind legs of female rats of the ACI/T strain. The tumors were removed about 2–3 weeks after inoculation.

Rat liver plasma membranes were isolated by the method of Neville [8]. Hepatoma membranes were isolated by the same method except that the tissue was homogenized in 2.8 mM citrate, instead of 1 mM $NaHCO_3$, as suggested by Emmelot et al. [9], to avoid disruption of the tumor nuclei. At the end of the preparation, the membranes were collected, washed (10 min centrifugation at 1200 × *g*) in 1 mM $NaHCO_3$ and stored at −20°C until used. After thawing, they were resuspended in 0.25 M sucrose, 5 mM Tris–HCl pH 7.4 at a protein concentration of 2–8 mg/ml.

The purity of the plasma membranes was checked by electron microscopy. In both preparations nuclei, mitochondria, lysosomes, microsomes, Golgi membranes and collagen fibers were absent. Spectrophotometric measurements could not detect the presence of cytochromes b_5 or P_{450}.

Determination of the number of binding sites and apparent dissociation constants were made by Scatchard plots constructed according to Azzi [10]. Protein concentration was determined by the method of Lowry et al. [11], using crystalline bovine serum albumin as a standard.

Fluorescence measurements were carried out in a Hitachi Perkin-Elmer spectrofluorometer Mod. MPF-2A.

ANS (from K&K) was used as magnesium salt after recrystallization.

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3. Results

Fig. 1a shows the fluorescence emission spectra of ANS free and bound to liver plasma membranes. The ANS fluorescence intensity in the presence of membranes was 3-fold higher and was accompanied by a blue shift from 520 to 500 nm. The presence of cations, Ca^{2+} and Mg^{2+}, produced a further increase of fluorescence and a further blue shift to 470 nm, suggesting ANS binding to more hydrophobic regions of the membrane, as a consequence of cation interaction.

Experiments with plasma membranes from Morris hepatoma (MHPM) 3924A were carried out in an attempt to identify structural differences between normal and tumor membranes. An emission spectrum of ANS in presence of Morris hepatoma plasma membranes (fig. 1b) indicated that the maximum fluorescence emission was blue shifted (about 15 nm) with respect to rat liver membranes.

Binding of ANS to plasma membranes was analyzed in terms of Scatchard plots. The data points were fitted by a single straight line; from the intercept of the abscissa the number of ANS sites on plasma membranes was calculated to be 47 nmol/mg protein (table 1),

Table 1
Effect of divalent cations on ANS binding to rat liver plasma membranes

Additions	Number of binding sites (nmol/mg protein)	K_D app. (μM)
none	47 ± 7 (5)	117 ± 15 (5)
5 mM $MgCl_2$	53 ± 3 (3)	24 ± 2 (2)
5 mM $CaCl_2$	50 ± 3 (3)	37 ± 5 (3)

Experimental conditions: plasma membranes from rat liver were suspended at a concentration of 0.1–0.3 mg/ml in 0.25 M sucrose 5 mM Tris-HCl pH 7.4. Data are obtained from Scatchard plots, constructed according to ref. [10]. The values are the mean ± S. E. M. (number of experiments)

with an apparent dissociation constant of 1.2×10^{-4}. The effect of Mg and Ca ions on the binding parameters of ANS, namely a decrease in the dissociation constant with no difference in the number of binding sites, was similar to what was found for mitochondrial membranes and red blood cell ghosts [6].

An effect similar to that obtained by divalent metal ions was observed by lowering the pH. The divalent

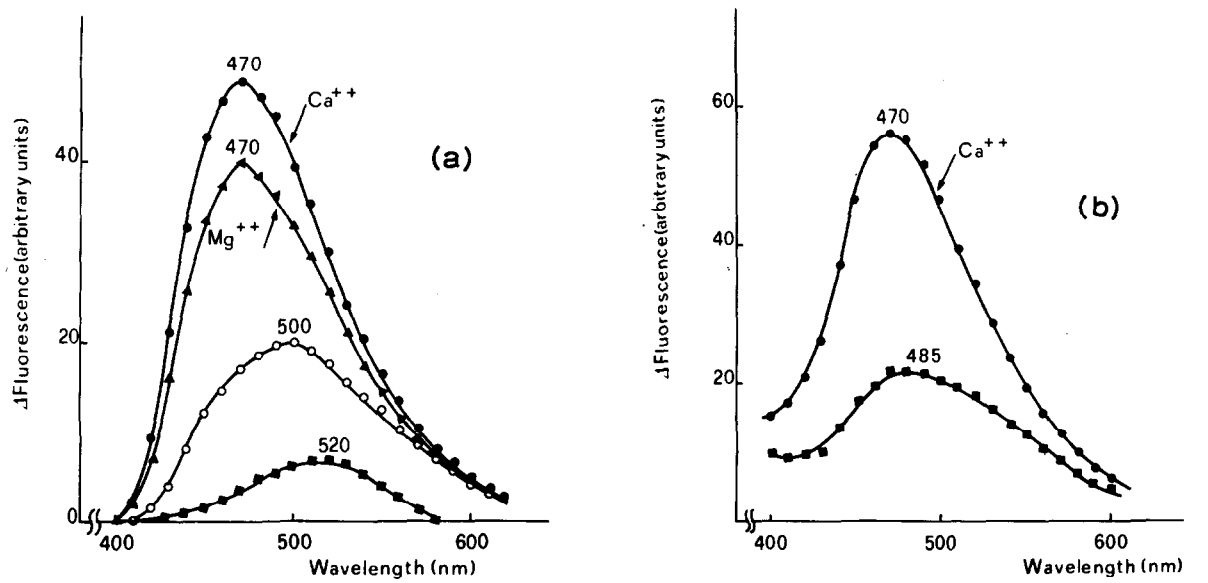

Fig. 1. Fluorescence emission spectra of ANS free, bound to liver plasma membranes and to Morris hepatoma plasma membranes. Rat liver plasma membranes (a) were incubated at a concentration of 0.12 mg protein/ml in 0.25M sucrose, 5 mM Tris–HCl, pH 7.4. The concentration of $MgCl_2$ or $CaCl_2$ was 5 mM. ANS was 5 μM. In (b) Morris hepatoma plasma membranes were incubated at a concentration of 0.10 mg protein/ml under the same experimental conditions.

cations and H^+ effects might be the consequence of the neutralization of negative charges on the membrane surface, allowing ANS binding to occur deeper, in less polar regions of the membrane. Such a conclusion is further strengthened by the observation that the ability of cations to increase the affinity of plasma membranes for ANS was greater for divalent than monovalent cations.

In Morris hepatoma plasma membranes the effect of Ca ions was very similar to that observed in liver plasma membranes. When a Scatchard analysis of the binding was carried out, it became evident that the presence of Ca^{2+} induced the appearance of extra binding sites for ANS with greater affinity (table 2).

No quantum yield changes were observed as a consequence of the interaction of divalent cations with plasma membranes either from tumor or from liver.

4. Discussion

Fluorescent probes do not afford absolute structural information but are extremely sensitive in comparing different membranes or a given membrane in different states. Therefore the study of structural effects of ions on plasma membranes or the study of the different behaviour of Morris hepatoma membranes do not go beyond the limitation imposed by this technique.

Evidence that ANS interacts with liver plasma membranes is afforded by the changes in spectral properties of the probe in the presence of membranes. In fact a 20 nm blue shift and an increase of approx. 3-fold in the fluorescence intensity is observed. This result may be immediately compared with that of Morris hepatoma where a larger blue shift is present (35 nm). Such a finding may be interpreted in terms of different characteristics of the ANS binding sites in the two types of membranes, those of tumors being more hydrophobic.

The effects of ions and pH on ANS fluorescence is consistent with shielding by the positive charges of the negative polar head groups of the membrane. The comparison between liver and Morris hepatoma membranes suggests that Ca^{2+} affects the hepatoma membranes not only by increasing the affinity for the probe, but also by increasing the number of binding sites. This result is consistent with unshielding of some membrane regions, as a consequence of the interaction with Ca^{2+}, which are scarcely accessible to ANS.

In conclusion, the interaction between ANS and plasma membranes suggests that the probe is going into rather hydrophobic domains. Divalent and monovalent cations modify the binding of the probe probably by ionic interactions with the membrane. The main difference of Morris hepatoma membranes with respect to membranes of rat liver, consists in a larger binding capacity for ANS to sites of lower polarity.

Table 2
Effect of Ca^{2+} on ANS binding to Morris hepatoma 3924A plasma membrane

Additions	Number of binding sites (nmol/mg protein)		K_D app. (μM)	
	low affinity	high affinity	low affinity	high affinity
none	110	–	85	–
5 mM $CaCl_2$	180	152	47	13

Experimental conditions: plasma were suspended at a concentration of 0.1 mg protein/ml in 0.25 M sucrose, 5 mM Tris–HCl pH 7.4. Data are obtained from Scatchard plots, constructed according to ref. [10].

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