RETINOL: A FLUORESCENT PROBE FOR MEMBRANE LIPIDS

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1. Introduction

Vitamin A, in particular all-trans retinol, affects a wide variety of biological membranes [1]. For example, low concentrations of retinol stabilise erythrocytes towards haemolysis, whereas higher concentrations induce gross membrane changes followed by haemolysis. The compound is surface-active, and will penetrate and expand phospholipid monolayers [2].

The theory of the absorption spectra of polyenes has been extensively treated [3] but much less is known about the fluorescence properties of these compounds. Although the fluorescence of all-trans retinol has been recognized for many years [4] it has only been studied recently from a theoretical standpoint [5]. In particular it has been noted that the O-O band intensity in emission is zero and that the radiative life-time calculated from the absorption and fluorescence spectrum is in dramatic disagreement with that derived from measurements of the quantum yield and mean life-time. These observations have been accounted for by the large change in nuclear configuration on excitation of polyenes of this type. This last property of retinol makes it a very suitable probe for polarity and environmental constraint [6].

2. Materials and methods

All-trans retinol was obtained from Sigma Chemical Co., the phospholipids from Koch and Light Laboratories. Eythrocyte stroma were prepared by the method of Dodge et al. [7]. Phospholipase *C* was from Sigma (Type I). Treatment of stroma with this enzyme was by the method of Lenard and Singer [8]. Phospholipid micelles were produced by evaporating a chloroform solution of the compounds under a stream of nitrogen, then taking up in aqueous buffer and sonicating at 20 kc for 1–2 min to obtain clear solutions. Concentrated stock solutions of retinol in absolute ethanol were stored under nitrogen at 0°. The sample solution (2 ml) was added to 0.02 ml of the stock retinol solution.

Fluorescence and fluorescence life-time measurements were carried out as described before [9].

3. Results

The absorption and corrected fluorescence spectra of retinol in cyclohexane had maxima at 325 and 520 nm respectively and were similar to those reported by others [5]. The position of these peaks varied very little in different solvents and in the membranes studied. Polarization of fluorescence (using polarized light for excitation) was 0.04 in ethanol but 0.35 in aqueous buffers. This observation suggests that retinol forms micelles (even at low concentrations) in water.

The fluorescence intensity of retinol is increased by the addition of lipids and erythrocyte stroma (fig. 1) reaching a maximum level corresponding to complete binding to the membrane. In all cases the uncorrected fluorescence excitation maximum was at 350 nm, corresponding to a red shift of about 5 nm relative to the excitation spectrum in ethanol. Table 1 summarises the fluorescence properties of retinol in the various systems (including different solvents) studied. For the properties of the bound probe all data were obtained from the flat portion of the binding curve (cf. fig. 1). Harmonic mean rotational relaxation times ρ_h were estimated from the Perrin equation [10] assuming $p_0 = 0.5$. Relative in-

Table 1
Fluorescence properties of retinol in solution, lipids and membranes.

System	Relative intensity	Quantum yield	τ (nsec)	τ_0 (nsec)	p	ρ_h (nsec)
Cyclohexane	1.8	0.020	5.0	250	0.04	0.8
Ethanol	1.0	0.011	3.5	320	–	–
Methanol	0.6	0.007	1.5	210	–	–
Aqueous buffer, pH 7.0	0.4	0.004	2.5	630	0.35	15
Stroma	1.3	0.014	10.0	700	0.35	58
Phospholipase *C*-treated stroma	–	–	8.0	–	0.30	30
Ovolecithin	1.0	0.011	7.0	630	0.26	19
Ovolecithin/ cholesterol (70:30 mole%)	1.2	0.013	8.0	600	0.30	30
Ovolecithin/lyso-lecithin (50:50 mole%)	1.0	0.011	7.0	630	0.26	19
Dipalmitoyl lecithin	1.2	0.013	7.0	530	0.30	26
Dipalmitoyl lecithin/ cholesterol (70:30 mole%)	1.7	0.019	11.0	580	0.40	110
BSA	3.6	0.04	9.0	230	0.40	90
Triton X-100	1.7	0.019	9.0	480	0.25	23

Lipid systems were at 500 μM total lipid. BSA at 2 mg/ml, Triton X-100 at 5 mM. Retinol 2 μM. All measurements were in isotonic NaCl, buffered at pH 7.0 with 10 mM phosphate at 25°, except for the organic solvents. The accuracy of fluorescence life-time measurements is ± 0.5 nsec.

tensities are corrected for lamp intensity variation with wavelength and correspond to excitation and observation at the corresponding spectral peaks.

Bovine serum albumin (BSA) was the only protein of those studied able to bind retinol strongly enough to break up the micelles in aqueous solution. Retinol reached maximum fluorescence within not more than 5 sec of mixing with stroma.

4. Discussion

Retinol interacts with phospolipid micelles, erythrocyte stroma and bovine serum albumin with an enhancement of its fluorescence and with a considerable increase in the fluorescence life-time. When the radiative life-time [τ_0] was calculated from the relation $\tau/Q = \tau_0$ it was found to vary between 200–600 nsec showing a large deviation from the theoretical value of τ_0 = 2.35 nsec obtained from the absorption spectrum. This deviation can be traced to the failure of the Strickler Berg equation used in calculating τ_0 to take into account the large change in nuclear configuration of the polyene on excitation [5]. This conclusion will lead to the prediction that a rigid environment in which the rearrangement in the nuclear framework is restricted should lead to a decrease in τ_0 derived from quantum yield and life-time measure-

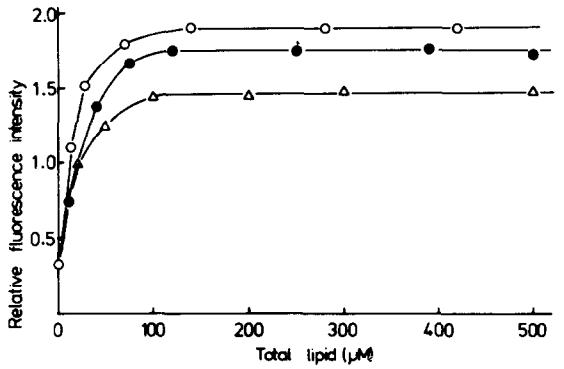

Fig. 1. The dependence of fluorescence intensity of retinol on the total lipid concentration of stroma and model lipid systems. Lipid content of stroma was calculated from data in [7], giving values of 60 μM cholesterol and 80 μM phospholipid in 0.1 mg/ml ghosts. Retinol concentration was 2 μM. Samples were in isotonic NaCl buffered at pH 7.0 with 10 mM phosphate, and at 25°.
○—○: stroma 0.1 mg/ml = 140 μM total lipid
●—●: ovolecithin/cholesterol 70:30 mole%
△—△: ovolecithin.

ments. Thus one can conclude that retinol is rigidly bound to BSA (in agreement with the polarization data) but that in the phospholipid micelles and erythrocyte stroma it has sufficient freedom to undergo rearrangement on excitation. The phospholipid micelles as prepared here are known to have average micellar weights in excess of 10^8 [11] and therefore the ρ_h values for these and for stroma must represent local mobility of the retinol probe with a correlation time of 10^{-7} to 10^{-8} sec in agreement with studies using lipid-soluble spin labels [12]. More interestingly retinol acts as a probe for the structure of the lipid systems. Thus the probe appears to be more mobile in the ovolecithin micelle (fatty acids in the fluid state at room temperature) than in the dipalmitoyl lecithin micelle (fatty acids in the ordered state at room temperature [13]). The presence of 30 mole percent cholesterol also causes an apparent increase in the local rigidity around the retinol probe as seen both in terms of the increased polarization and increased τ, in agreement with conclusions from studies with spin labels [14]. The fluorescence properties of retinol in erythrocyte stroma are very similar to those of retinol in the lipid micelles. It is of particular interest that the value of τ_0 is high (unlike on binding to BSA) suggesting mobility in the system that could not be easily deduced from polarization measurements alone. Treatment of erythrocyte stroma with phospholipase *C* increases the mobility of the probe as seen from the reduced polarization and decreased τ.

Polyenes thus provide a new type of probe for membrane "fluidity" by a combination of polarization and fluorescence life-time measurements.

Acknowledgements

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