RETINOID-PHOSPHOLIPID INTERACTIONS AS STUDIED BY MAGNETIC RESONANCE

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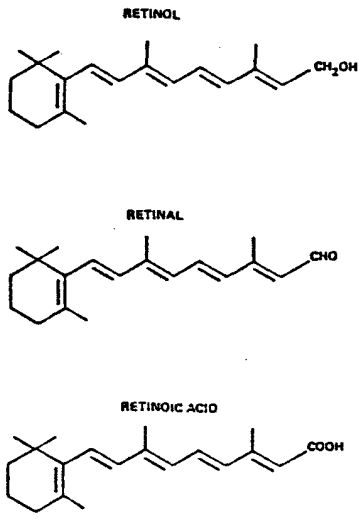
I. INTRODUCTION

The retinoids (vitamin A and derivatives) - retinol (vitamin A), retinal (vitamin A aldehyde) and retinoic acid (vitamin A acid) - form a homologous series of amphiphilic molecules (Figure 1). They vary in structure only at the polar end but differ markedly in their essential support of biological function (1). At high doses they become toxic, which may be due to membrane disruption.

Figure 1. Retinoids.



Earlier work has shown that retinoids perturb membrane properties such as permeability, phase behaviour, order and microviscosity (2-5). The present studies are concerned with the application of magnetic resonance techniques to the elucidation at the molecular level of the interactions of all-trans retinoids with phospholipid model membranes.

ESR of nitroxide spin labelled fatty acids intercalated into multilamellar phospholipid liposomes enabled the influence of each retinoid on acyl chain order and mobility to be monitored throughout the membrane. The effect of retinoid incorporation on spin lattice relaxation of individual resonances in high resolution ^{1}H decoupled ^{13}C NMR spactra recorded for unilamellar phospholipid vesicles was also investigated. In contrast to multilamellar liposomes, which are typically 10,000 Å in diameter, unilamellar vesicles produced by sonication are approximately 200 Å in diameter and tumble sufficiently quickly in aqueous solution to give rise to high resolution spectra (6).

II. EXPERIMENTAL

A. ESR

ESR spectra (9.2 GHz) for 5-,7-,10-,12- and 16-doxyl stearic acids intercalated at low concentration (1 mol%) into dipalmitoylphosphatidylcholine (DPPC) multilamellar liposomes (1% w/v in 10 mM phosphate/1mM EDTA buffer, pH 7.5) were recorded at 50°C as a function of retinoid content. Order parameters S were calculated using

$$S = \frac{T_{\parallel} - T_{\perp} - C}{T_{\parallel} + 2T_{\perp} + 2C}(1.66)$$

where $T_{\parallel}$ and $T_{\perp}$ are the apparent parallel and perpendicular hyperfine splitting parameters, the constant $C = 1.4 - 0.053(T_{\parallel} - T_{\perp})$ is an empirical correction for the difference between the true and apparent values of $T_{\perp}$, and the factor 1.66 is a solvent polarity correction factor (7). Calculation is restricted to the upper portion of the fatty acid chain (5, 7 and 10-positions), where molecular motion is anisotropic enough to produce spectra for which outer and inner hyperfine extrema are defined. The order parameter can take values in the range $0 \leqslant S \leqslant 1$, the respective limits representing isotropic motion and axial motion with no eff-axis flexing. In the lower portion of the chain (12- and 16-positions), where molecular motion is approximately isotropic, correlation times τ_c were estimated from spectra characteristic of high disorder on the assumption of isotropic motion according to

$$\tau_c = 6.5 \times 10^{-10} W_o \left[\left(\frac{h_o}{h_{-1}} \right)^{1/2} - 1 \right]$$

where W_o is the peak to peak width of the central line, and h_o/h_{-1} is the ratio of the heights of the central and high field lines, respectively (8).

B. ^{13}C NMR

^{1}H decoupled ^{13}C NMR spectra (75.46 MHz) were recorded at 35°C for unilamellar vesicles of egg phosphatidylcholine (egg PC, 20% w/v in 100 mM phosphate buffer, pH 7.5) in the absence and presence of retinoid. Decoupling was gated on only during data aquisition and spin lattice relaxation times were measured by the optimized inversion recovery sequence (9).

III. RESULTS

A. ESR

The effects as a function of concentration of retinoids on acyl chain motion in DPPC bi-

Figure 2. Order parameter vs. retinoid concentration in DPPC bilayers at 50°C.

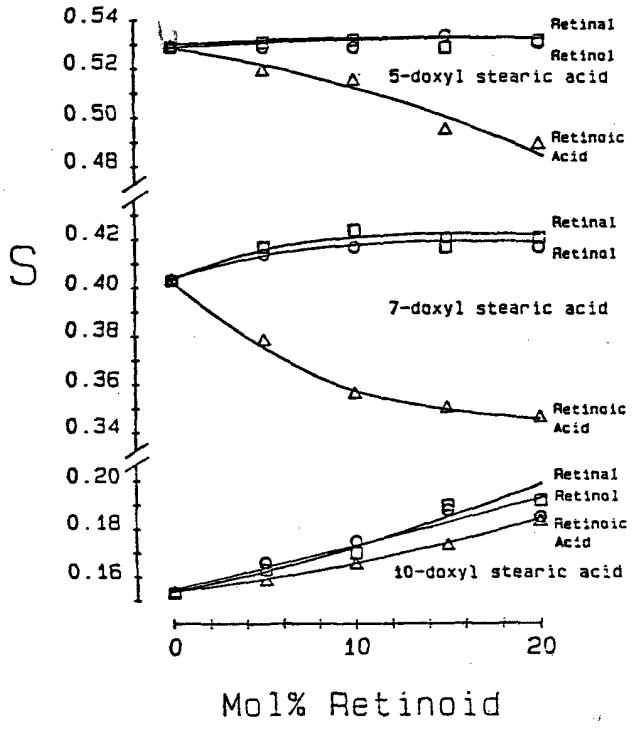
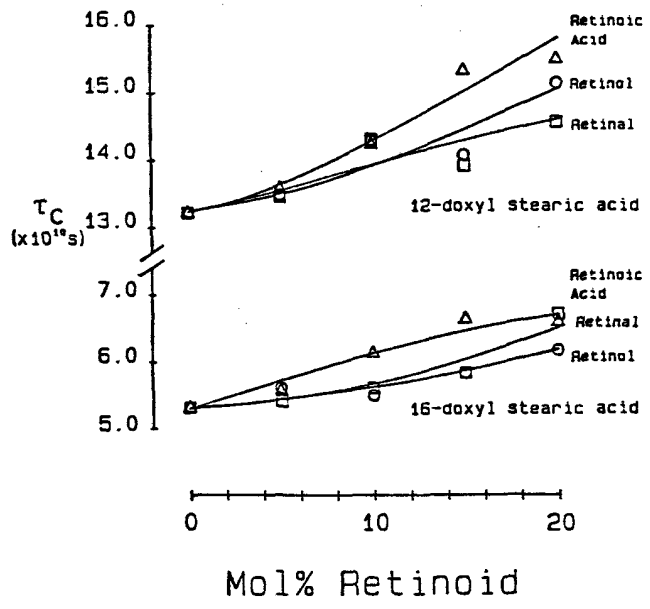


Figure 3. Correlation time vs. retinoid concentration in DPPC bilayers at 50°C.

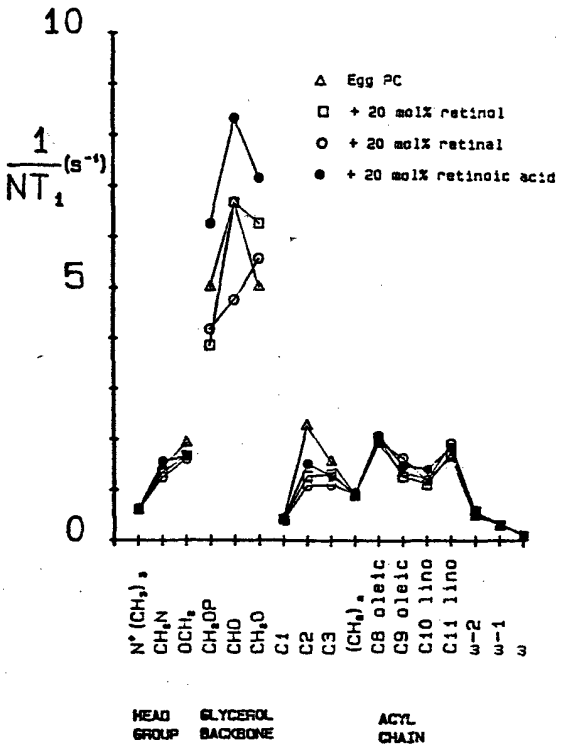


layers, as monitored by ESR of spin labelled stearic acids, are summarized in Figures 2 and 3. Retinol and retinal exhibit similar behaviour. They have little influence in the upper portion of the chain (positions 5 and 7), whereas in the lower portion order parameters (position 10) and correlation times (positions 12 and 16) are increased by as much as, respectively, 25% and 15%. In contrast, retinoic acid disorders the upper part of the chain by as much as 12% but affects the lower part in a similar manner to retinol and retinal.

B. ^{13}C NMR

Spin lattice relaxation rates $1/NT_1$, normalized with respect to the number of directly bonded protons N, are shown in Figure 4 for egg PC vesicles in the absence and presence of 20 mol% retinoid. No retinoid-associated trend is discernible.

Figure 4. ^{13}C NMR relaxation rate as a function of position in egg PC vesicles at 35°C.



IV. DISCUSSION

The ESR results indicate order in the upper portion of the lipid chain is essentially unaffected by retinol and retinal. Farther along the chain order parameters and correlation times are increased by these retinoids. Space filling models suggest such a profile is physically plausible since the bulky hydrophobic cyclohexene ring would be expected to locate, and hence restrict acyl chain motion, towards the centre of the bilayer.

Retinoic acid, in contrast, disorders the upper part of the lipid acyl chain. Lower down the chain it restricts acyl chain motion to a similar extent

terms of the strongly hydrophilic nature of the carboxylic group. This, we contend, would locate retinoic acid higher within the membrane than retinol or retinal and lead to a disruption of acyl chain packing near the region of the aqueous interface. Consistent with our explanation, ESR spin label experiments with DPPC membranes show decanol has negligible effect on lipid chain motion while decanoic acid decreases order (10).

No clear trend in retinoid influence is apparent in the ^{13}C NMR spin lattice relaxation times. Possible reasons for the discrepancy with the ESR data include: retinoid packing differs in the bilayer of highly curved vesicles and liposomes (11); the timescales of ESR and ^{13}C NMR are different; and the effects seen by ESR are too subtle to be observed in ^{13}C NMR T_1 values.

V. CONCLUSION

A distinction between the effects on membrane acyl chain motion of retinoic acid and of retinol and retinal is established by the ESR spin label data. Retinol and retinal restrict acyl chain motion near the centre of the phospholipid bilayer and have little effect towards the aqueous interface. Retinoic acid similarly restricts acyl chain motion approaching the centre of the membrane but, unlike retinol and retinal, disorders the bilayer in the upper portion of the chain. ^{13}C NMR T_1 values for sonicated vesicles show no perceptible pattern in retinoid influence on phospholipid molecular motion. Support for the difference in retinoid induced change to membrane properties seen by ESR is, however, provided by permeability measurements in which the rate of liposome swelling in isotonic erythritol was monitored by optical absorbance (12). These measurements demonstrate that retinoic acid greatly enhances permeability, whereas retinol and retinal affect permeability only slightly (13).

Acknowledgements

It is a pleasure to thank Professors Marvin D. Kemple and B. D. Nageswara Rao for use, respectively, of an IBM/Bruker ER 200D X-band ESR spectrometer and of a Nicolet (GE) NT-300 FT NMR spectrometer.

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