Ion Binding in Liquid Crystals Studied by NMR. III.* ^{23}Na Quadrupolar Effects in a Model Membrane System

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Almost important problem in membranology is the elucidation of the mechanism of the binding of small molecules or ions (*e.g.* K^+ and Na^+, but also the divalent Ca^{2+} and Mg^{2+}) to membrane surfaces. The progress in this field has, however, been retarded by the difficulties encountered in finding good nondestructive direct methods for the study of these phenomena. Since all the ions mentioned have nuclei with non-spherical charge distributions it should be possible to use their electric quadrupole moments to trace ion-membrane interactions. In this communication the applicability of quadrupolar effects in ^{23}Na nuclear magnetic resonance to the study of sodium ion binding to membranes will be discussed. As is demonstrated by the results this method constitutes a promising possibility for obtaining deeper insight into membrane function.

It is well known that phospholipids are important constituents of cellular membranes.[1] As a simple membrane model system the lamellar liquid crystalline phase in the ternary system lecithin-sodium cholate-water was chosen. The phase diagram for this system has been determined by Small *et al.*[2] A typical continuous-wave ^{23}Na NMR spectrum of the lamellar mesophase is represented in Fig. 1. In contrast to isotropic solutions where only one ^{23}Na signal can be observed the present case gives a strong splitting into three peaks.

The ^{23}Na atomic nucleus, with a spin quantum number equal to 3/2, possesses an electric quadrupole moment which interacts with electric field gradients at the nuclear position. If all of the orientations of the quadrupole moment with respect to the lamellae are not equally probable, a so-called quadrupole splitting of the resonance signal will result.[3] In the present case the splitting is due to first-order quadrupolar effects, which is evident from the spectral shape (relative intensities and symmetry) and the invariance of the magnitude of the splitting to magnetic field variations. Since a splitting is actually observed we may conclude that the sodium ions are interacting strongly with the model membrane surfaces. This is in contrast with some other lamellar mesophases.[4-6] The magnitude of the splitting, theoretical expressions of which are given in Ref. 7, depends on the electric field gradients, on the degree of motional anisotropy, and on the fraction of sodium ions bound to the surfaces. The basic principles underlying this method are more thoroughly presented in Ref. 8.

Another interesting possibility to study ion binding to mesophases is to measure the

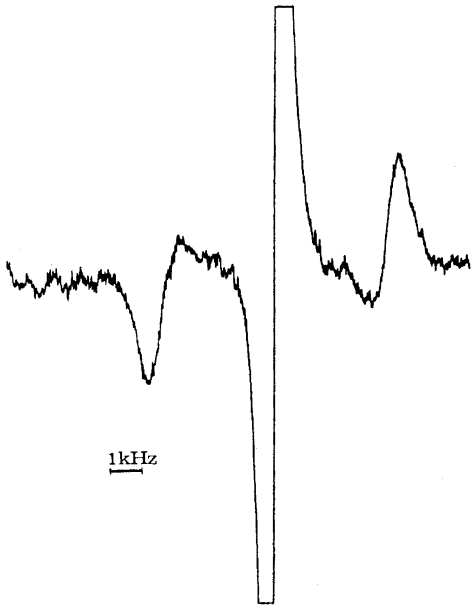

Fig. 1. ^{23}Na NMR spectrum of a lamellar liquid crystalline sample with the composition (by weight) 64 % lecithin, 16 % sodium cholate, and 20 % water. The spectrum was obtained at 25 ± 2°C and at a resonance frequency of 15.82 MHz. The separation between the outer peaks amounts to 8.4 kHz. (The intense central peak cannot be displayed in full at the amplification employed.)

* For part II, see Ref. 8.

nuclear quadrupole relaxation times. By the conventional $\pi - \pi/2$ pulse sequence the spin-lattice relaxation time, T_1, of ^{23}Na was determined for a sample with the composition (by weight) 64 % lecithin, 16 % sodium cholate, and 20 % water. Since the magnetization decays exponentially with the pulse spacing, one T_1 suffices to describe the experiments. It was found that $T_1 = 1.33 \pm 0.05$ ms, which is an order of magnitude less than the value observed in an aqueous sodium chloride solution ($T_1 =$ 60 ms). Thus a strong interaction between the membrane surfaces and the sodium ions is evident also from this measurement.

Of course, the binding of sodium ions to membrane surfaces is a well-known phenomenon. The details of this interaction are, however, not known and the reason for this is above all a lack of suitable experimental methods. The method proposed in this communication presents several advantages, *e.g.* the negligible perturbation of the system studied and the sensitivity to small changes in interaction strengths. From this preliminary study it may be concluded that it should be possible to use small cations as NMR probes for the examination of interactions close to the membrane model surface. We are currently attempting to investigate certain aspects of ion binding to membrane models by this method, *e.g.* the competition between sodium and other alkali ions and the effect of cholesterol on ion binding.

Experimental. The lecithin was extracted from egg yolk and purified according to the procedure described by Singleton *et al.*[9]

The lamellar mesophase was prepared by adding water to a mixture of sodium cholate and lecithin kept under nitrogen atmosphere. One sample was also prepared from commercially available lecithin (from BDH) and was found to give essentially the same NMR spectrum as the sample obtained from lecithin prepared in our laboratory. The continuous-wave ^{23}Na NMR spectrum was recorded with a Varian V-4200 NMR spectrometer as described elsewhere.[8] The T_1 measurements were performed at 25°C and 23.81 MHz with a Bruker B-KR 322 s pulsed NMR spectrometer.

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Isoelectric Fractionation, Analysis, and Characterization of Ampholytes in Natural pH Gradients. II. Buffering Capacity and Conductance of Isoionic Ampholytes. A Correction

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In the article [1] of the above title published in 1962, there is, unfortunately, a physico-chemical inconsistency which the author has felt irritating ever since; yet its practical importance has not been considered great enough to warrant a correction in the scientific press. The method of isoelectric focusing, however, is now in a state of rapidly growing importance, and