Natural Abundance ^{13}C Nuclear Magnetic Resonance Spectra of the Lipid in Intact Bovine Retinal Rod Outer Segment Membranes†

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ABSTRACT: Natural abundance ^{13}C nmr spectra for intact bovine retinal rod outer segments have been recorded. Resonances due only to the lipid components of the disk membranes were observed and these resonances have been assigned. Presumably resonances due to rhodopsin and other proteins are not observed due to their low intensity and broad line widths. ^{1}H nuclear magnetic resonance spectra of intact disks were extremely broad with little definition; ^{13}C T_1 values for the lipid resonances were measured for both unbleached and bleached rod outer segments. No changes were observed for the T_1 values on bleaching, nor were any chemical-shift changes observed. These findings are discussed in terms of the role played by the lipid phase in functional properties of rod outer segments.

Rod cells of the vertebrate retina are the photoreceptor cells for black and white vision. The outer segment of these cells is composed of densely packed, free-floating, biconcave, or disk, membranes. These specialized membranes contain about equal amounts of protein and lipid on a dry weight basis, about four-fifths of their protein being present as the visual receptor protein rhodopsin. The disk membrane lipids provide a highly fluid environment in which the protein molecules are capable of rapid rotational motion (Cone, 1972; Brown, 1972). Most of the lipids are the phospholipids phosphatidylethanolamine, phosphatidylcholine, and phosphatidylserine. An unusual feature is their side-chain unsaturated fatty acid composition, which is among the highest reported for biological membranes (Nielson *et al.*, 1970). Saturated fatty acids account for about 95% of the fatty acid composition at the number 1 position of these phospholipids, while the highly unsaturated fatty acid decosahexenoic acid (C 22:6) makes up about 70% of the fatty acid composition at the number 2 position (Anderson and Sperling, 1971). It has recently been found that resolved ^{13}C nuclear magnetic resonance (nmr) spectra can be obtained from pure phospholipid vesicles, obtained by sonication of dispersions, which yield only poorly resolved ^{1}H nmr spectra (Levine *et al.*, 1972a) and that information about the molecular motion of the lipids can be obtained from the ^{13}C spin–lattice relaxation time T_1. We have studied the ^{13}C and ^{1}H nmr spectra of bovine rod outer segments in both the unbleached and bleached states. The preparations from fresh bovine eyes consist mostly of intact rod outer segment disk membranes (McConnell, 1965).

Materials and Methods

Bovine rod outer segments were isolated using the method of McConnell (1965) as modified by D. S. Papermaster (personal communication, 1972). Preparations were made from both fresh bovine eyes obtained from a local slaughterhouse, and frozen dark-adapted bovine retinas from Geo. Hormel Co., Austin, Minn. If the rods were not used immediately, they were stored frozen in the dark at −20°. Argon was used throughout the preparation and measurements to retard lipid oxidation. Docosahexenoic acid was obtained from the Hormel Institute, Austin, Minn. ^{13}C nmr spectra of both bleached and unbleached rod outer segments were obtained on a Varian XL-100 spectrometer operated at 37°. Proton spectra were taken on a Varian 220-MHz spectrometer. ^{13}C T_1 measurements were obtained by the progressive saturation technique (Freeman and Hill, 1971).

Results

The natural abundance ^{13}C nmr spectrum of unbleached bovine rod outer segments at 37° is shown in Figure 1a. Transients (30,000) were accumulated and Fourier transformed to obtain this spectrum. The ^{13}C nmr spectrum of docosahexenoic acid in deuterated chloroform is presented in Figure 1b. Assignments were arrived at from the relative intensities of the resonances in the free acid. The two strongest resonances in the rod outer segment spectrum can be assigned to the vinyl and methylene resonances of docosahexenoic acid. Choline methyl resonances are also present, as well as the methylene and terminal methyl resonances of the saturated fatty acids. The T_1 values are shown in Table I. The low signal-to-noise ratio of the spectra led to the relatively large errors shown. Results were consistent with a single T_1 relaxation time for each resonance, although a small fraction of the resonance intensity decaying at a different rate cannot be ruled out because of the low signal-to-noise ratio. There was no difference in the spectrum or T_1 values between preparations obtained from fresh eyes, and from frozen retinas. Bleaching the rod outer segments led to no observable change in the ^{13}C spectrum. Neither the line positions nor the T_1 values changed, within experimental error.

The 220-MHz proton nmr signal from the same preparations consisted of very broad overlapping resonances which could not be resolved. The terminal methyl resonance had a line width of 100 MHz, while all the other resonances were broader.

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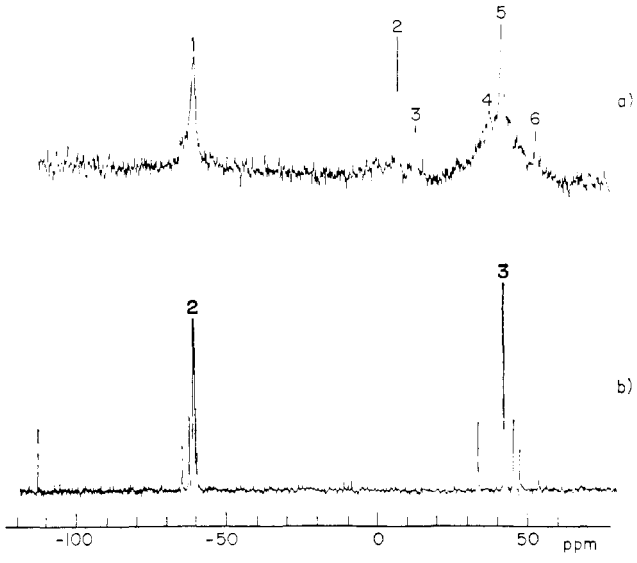

FIGURE 1: (a) ^{13}C nmr spectrum of bovine rod outer segments at 37°, Tris-Mg^{2+} buffer, pH 7.5; 15,000 scans, in parts per million from dioxane: (1) $(\overset{*}{C}H{=}CH)$; (2) Tris buffer; (3) $N^{+}(\overset{*}{C}H_3)_3$; (4) $(CH_2)_n$ region for saturated fatty acids; (5) $(CHC\overset{*}{C}H_2CH{=}C)$; (6) $\overset{*}{C}H_3$. (b) ^{13}C nmr spectrum of docosahexenoic acid at 30°: (1) COOH; (2) (CH=CH) region; (3) $(CH{=}C\overset{*}{C}H_2CH = CH)$.

Discussion

The line widths and T_1 values of the ^{13}C resonances of phospholipid membranes are an indication of the fluidity of the membrane (Levine *et al.*, 1972a,b). The present nmr results substantiate that the rod outer segment disk membrane is one of the most fluid biological membranes, consistent with its known high content of unsaturated fatty acids. The only other biological membrane which has been studied to date by ^{13}C nmr is the sarcoplasmic reticulum membrane (Robinson *et al.*, 1972) which also has a large fraction of unsaturated fatty acids and yields a well-resolved spectrum. Artificial phosphatidylcholine vesicles containing saturated side chains yield well-resolved ^{13}C spectra only at high temperatures above the phase transition (Levine *et al.*, 1972a).

The long T_1 value for the docosahexenoic acid methylene carbons in the rod outer segment ^{13}C spectrum indicates that this fatty acid in particular undergoes considerable conformational motion in the membrane. The considerable broadening of the proton resonances compared to the ^{13}C resonances might indicate interaction with the protein, mainly rhodopsin, which would have a larger effect on the proton resonances because the intermolecular proton–proton distances are smaller than the intermolecular proton–carbon distances.

It is rather surprising that there is no detectable change in the line widths or T_1 values of the resonances upon bleaching the rod outer segments. Apparently the bleaching of rhodopsin in the rod outer segment disk membrane, the initial visual event preceding the signal amplification and conduction events, does not cause detectable changes in the membrane structure as a whole. Such a finding, however, does not rule out a more localized structural change in rhodopsin which would not affect the bulk of the membrane lipid. It is known that rhodopsin binds lipid strongly but that a large amount of lipid may be extracted from disk membranes without disrupting membrane integrity or changing the visible spectrum of rhodopsin (Poincelot and Abrahamson, 1970; Borggreven *et al.*, 1972; Dratz *et al.*, 1972). Structural changes in a small fraction of the total lipid probably would not have a measurable effect on the lipid ^{13}C T_1 value at the error limits we were able to achieve.

TABLE I: ^{13}C T_1 Values of Unbleached Rod Outer Segments at 37°.

Resonance	T_1 (sec)
$N^{+}(\overset{*}{C}H_3)_3$	0.4 ± 0.2
$-\overset{*}{C}H_2CH-$	0.35 ± 0.05
$C{=}CHC\overset{*}{H}_2CH{=}C$	1.0 ± 0.1
$(\overset{*}{C}H_2)_n$ (saturated)	0.3 ± 0.1
$\overset{*}{C}H_3$	>3

It does seem likely, however, that the large structural change proposed by Blasie (1972), in which rhodopsin sinks a distance one-third of its radius into the membrane upon bleaching, would be detected by our measurements.

In conclusion, it is encouraging that well-resolved ^{13}C natural abundance nmr spectra can be obtained from an intact biological membrane, and that this technique can then be employed in the study of membrane properties at the molecular level.

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