SHORT COMMUNICATIONS

BBA 73057

Nuclear resonance studies of erythrocyte ghosts at 220 Mcycles/sec

In previous communications[1,2], we have reported that intact erythrocyte ghosts or water-washed stroma gave weak and ill-defined high resolution proton magnetic resonance (PMR) signals and that a consistently reproducible spectrum was observed only after sonic dispersion of these materials. The assignments of spectral peaks and our interpretations of the observed line broadenings or narrowing have been discussed in detail in these communications. The changes in spectra caused by detergents, sodium deoxycholate and lysolecithin were also discussed. However, a number of points remain unanswered in this work, *e.g.* to what extent can one extrapolate the results obtained with sonicated dispersions to the situation in native membrane? Under our experimental conditions, sonication reduces intact ghosts to particles of microsomal dimensions which can be sedimented in the ultracentrifuge. Electron microscopy of the sedimented material after glutaraldehyde and osmium tetroxide fixation and staining according to the method of SABATINI, BENSCH AND BARRNETT[3], shows particles (<2000 Å in radius) bounded by layered structures some 80 Å thick, whereas unsonicated ghosts appear as large sheets of membrane. (These results are in agreement with the data of FINEAN *et al.*[4].)

In the present communication, however, we report observations made on intact non-sonicated erythrocyte ghosts with high-frequency 220 Mcycles/sec high resolution PMR spectroscopy. Haemoglobin free erythrocyte ghosts were prepared from human erythrocytes by methods described previously[5]. They were equilibrated with ^2H_2O by ultracentrifugation in 20 mosM phosphate buffer (pH 7.4) in 99.7 % ^2H_2O twice at 25000 rev./min for 2 h and lastly at 25000 rev./min for 16 h to yield a compact pellet. This pellet was resuspended in a small volume of phosphate buffer in ^2H_2O (20 mosM, pH 7.4) and dialysed against the same buffer for 4 h. The final suspension had a protein concentration of about 18 mg/ml as determined by the ninhydrin procedure[6]. Aliquots of this suspension were solubilised separately by addition of sodium deoxycholate, sodium lauryl sulphate and lysolecithin at a concentration of 1 mg/ml membrane protein. Controls of these solubilising agents were prepared in ^2H_2O buffered to 20 mosM phosphate (pH 7.4). These samples were examined on a 220 Mcycles/sec Varian Associates high resolution spectrometer with a polarizing field of 52000 Gauss furnished by a superconducting solenoid.

Spectra were obtained for solubilized samples and their controls between 0–10 ppm in a single scan, with 2,2-dimethylsilapentane 5-sulphonate as internal reference standards at 10 ppm. For intact ghosts in buffered ^2H_2O, spectra were obtained between 0 and 20 ppm with a sweep width of 2500 cycles/sec on either side of H^2HO

Abbreviation: PMR, proton magnetic resonance.

peak (10 ppm) and also with a sweep width of 5000 cycles/sec on the right hand side of H^2HO peak where structural features were apparent. A sample of intact ghosts in buffered ^2H_2O was also examined on a 60 Mcycles/sec Perkin Elmer R10 spectrometer after several accumulations (between 500 and 1100) with a CAT (Computer of Averaging Transients). A sweep width of 100 ppm on either side of H^2HO peak was employed). These were recorded without spinning the sample. High resolution spectra were also obtained on the 220 Mcycles/sec spectrometer between 0 and 10 ppm after sonic dispersion of intact ghosts as described earlier[2].

PMR spectra of these 'intact' ghosts at 60 Mcycles/sec and 220 Mcycles/sec are shown in Figs. 1a and 1b. The 60 Mcycles/sec spectrum is very broad with no distinct features of fine structure. The half-height line width cannot be measured accurately owing to the tailing of the broad line. It is estimated to be approx. 1000 cycles/sec. At 220 Mcycles/sec a very weak absorption occurs at 6.2–6.4, 6.7, 7.9 and 9.1 ppm at 40°. These are superimposed on a fairly broad component between 5 and 10 ppm. There is no detectable improvement in resolution at 60°. The broad component becomes distinguishable after a sweep-width of 5000 cycles/sec (between 5 and 20 ppm). This is shown in Fig. 1b. It is interesting to note that the half-line width between 7 and 9 ppm is about 450 cycles/sec. This is the region where hydrocarbon chains of the lipid at 8.7 are unresolved and where major absorption band in globular proteins occurs. Superimposed on this band are signals at 7.9 due to $N–CO–CH_3$ or CH_2 groups of some amino acids of membrane proteins (*e.g.* glutamic acid) and 9.1 due to CH_3 protons. There is also a weak absorption at 6.7 ppm due to $N(CH_3)_3$ protons. The sharp signal at 6.4 ppm is questionable and appears to be associated with adventitious

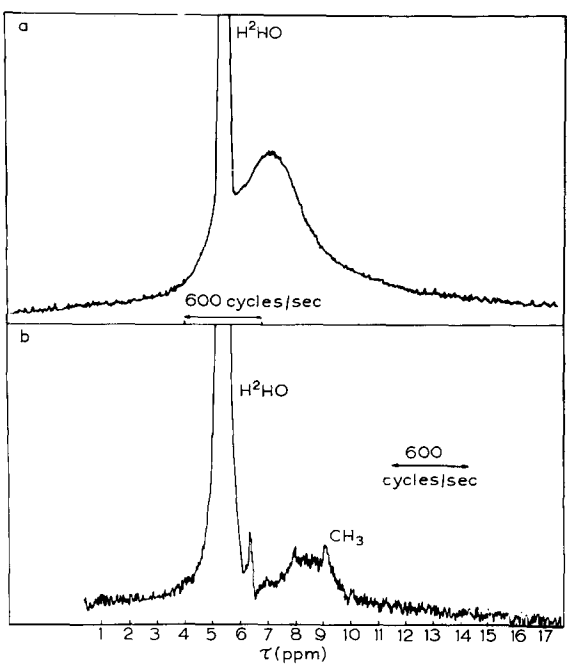

Fig. 1. a. PMR spectrum of intact erythrocyte ghosts in 20 mosM sodium phosphate buffer (pH 7.4) in ^2H_2O on 60 Mcycles/sec high-resolution spectrometer (1024 scans) at 40°. b. PMR spectrum of the ghosts in buffered ^2H_2O on 220 Mcycles/sec Varian Instrument at 40° (single scan).

impurities (coming either from the dialysis bag or from the blood preservative) but the broad absorption below this is genuine and has been observed in the 60 Mcycles/sec spectrometer on freeze-dried material repeatedly washed with ^2H_2O. These signals are similar to those observed with sonicated membranes and reported in our earlier paper[2]. The spectrum of sonicated membrane at 220 Mcycles/sec is shown in Fig. 2.

Spectra obtained with intact ghosts on solubilisation with sodium deoxycholate and sodium lauryl sulphate show that a narrowing of the $(CH_2)_n$ signal at 8.7 ppm

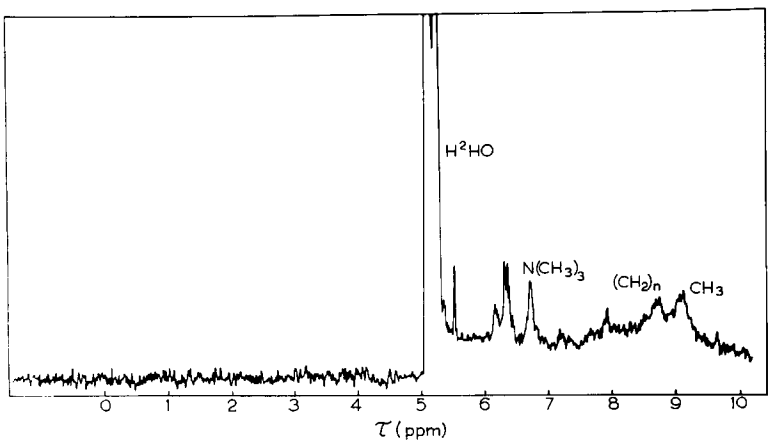

Fig. 2. High-resolution PMR spectrum of erythrocyte ghosts in buffered ^2H_2O after sonic dispersion at 40° on 220 Mcycles/sec spectrometer (single scan).

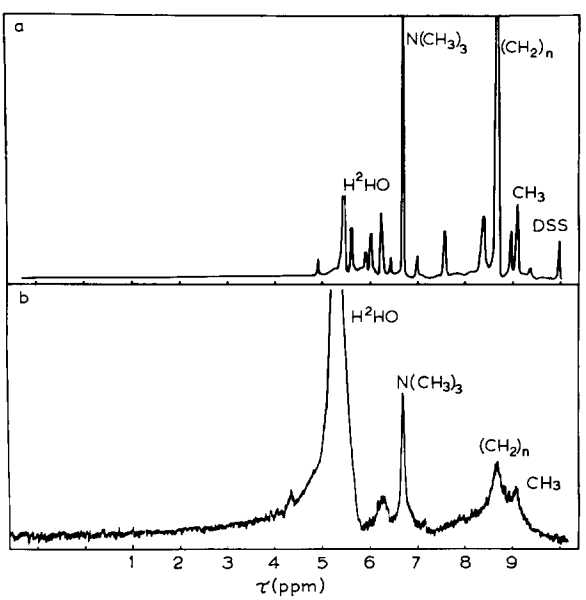

Fig. 3. a. A high-resolution spectrum of 1.75 % lysolecithin (Sigma) in 20 mosM phosphate buffer in ^2H_2O (pH 7.4). DSS = dimethylsilapentane 5-sulphonate. b. Spectrum of lysolecithin-solubilised erythrocyte ghosts at the same concentration of lysolecithin in buffered ^2H_2O (pH 7.4). The spectra are recorded on the 220 Mcycles/sec instrument (single scan) at 40°.

is produced. There is a peak due to unsaturation at 4.7–4.8 ppm and splitting of some of the signals. With lauryl sulphate an additional absorption at 2.5–2.8 ppm due to aromatic side chains of the membrane protein is observed. The spectrum of the intact ghosts solubilised by lysolecithin shows a marked inhibition of the $(CH_2)_n$ signal of lysolecithin itself (Fig. 3). These results provide support for our previously published 60 Mcycles/sec spectroscopic observations on sonicated erythrocyte membrane fragments and their co-dispersions with other surfactants.

A particular feature of the use of the 220 Mcycles/sec instrument is that, because of its excellent signal to noise ratio, good spectra are observed with only a single scan, whereas computer averaging for many hours is required with the 60 Mcycles/sec instrument. We are currently examining by broadline PMR spectroscopy and other physical methods, intact ghosts, their total lipids and phospholipids to obtain further insight into the nature of lipid protein interactions.

We acknowledge the Science Research Council, Dr. J. K. BECCONSALL and the staff of I.C.I. Runcorn for allowing us the use of the Varian 220 Mcycles/sec instrument.

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Received July 15th, 1968