A NUCLEAR MAGNETIC RESONANCE STUDY OF SODIUM ION INTERACTION WITH ERYTHROCYTE MEMBRANES

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SUMMARY

Sodium-23 nuclear magnetic resonance line widths have been used to study sodium ion interaction with erythrocyte membranes and erythrocyte membrane proteins. Detergent solubilization has been effective in freeing sodium and potassium binding sites which are not available for rapid chemical exchange with the whole membrane. Evidence for sodium ion binding has been obtained by showing that the resonance line width increases in the presence of solubilized membrane. Competition by potassium for binding sites is demonstrated by a decrease of the sodium line width in the presence of potassium ion.

Nuclear magnetic resonance investigations of sodium-23 ions have previously been used to study sodium-EDTA complexes[1] and also sodium ion-RNA complexes.[2] This work was conducted using pulse techniques to measure T_1, the spin-lattice relaxation time. Earlier, sodium in muscle tissue had been investigated; however, no resonance was detected for sodium bound to muscle.[3] We wish to report a study of sodium-23 interaction with erythrocyte ghosts using nmr. The line width of the absorption signal at half height was measured as previously reported for chloride ion by Stengle and Baldeschwieler.[4] Under conditions of fast chemical exchange among all the quadrupolar ion species, the observed line width, $\Delta\nu$, at half height is just a weighted average of all the forms

$$\Delta\nu = \Delta\nu^{free} \; X^{free} + \sum_i \Delta\nu_i^{bound} \; X_i^{bound}$$

$\Delta\nu^{free}$ is the free hydrated sodium ion line width and $\Delta\nu_i^{bound}$ is the bound sodium line width, where i indicates the possibilities of numerous binding sites. X represents the respective mole fractions. This work was under-

taken to determine whether or not there are binding sites on the membranes for sodium ion which allow rapid exchange with free sodium ion and, therefore, lead to an observable line broadening in the nmr signal.

EXPERIMENTAL

The sodium-23 line widths were obtained at 15.879 MHz and field strength of 14,000 Gauss; the chlorine-35 line widths were obtained at 5.878 MHz; A modified Varian DP-60 using 2000 Hz modulation and a lock-in amplifier for base line stabilization was used in conjunction with a voltage controlled General Radio Model GR 1164 frequency synthesizer.[5] The radio frequency was swept and frequency values recorded at several points in the spectral scan. The observed line widths are reported as the frequency difference at half height for the absorption mode signal.

Spectra were taken of various 0.16 M sodium chloride solutions containing erythrocyte ghosts prepared by a slightly modified method of Dodge, Mitchell, and Hanahan.[6] To the ghost suspensions were added small aliquots of detergent or potassium chloride.

Fractionation of erythrocyte ghosts on Bio-Gel P-300 in 0.3% (w/v) sodium dodecyl sulfate (SDS), 0.16 M sodium chloride at pH approximately 10 was carried out to separate the membrane components. The figure shows the separation and the subsequent combination of fractions. The fractions were dialyzed initially against water at pH>9, followed by exhaustive dialysis against glass distilled water. The insoluble protein was removed by centrifugation and the soluble protein solution was lyophilized.

RESULTS

The sodium-23 line width in 0.16 M sodium chloride is 12±1 Hz. Some of this line width is probably from field inhomogeniety as coarse adjustments on the homogeneity controls could broaden the line width. All values reported were obtained with field homogeneity set so that a sodium line width of 12±1 Hz was obtained for the 0.16 M standard.

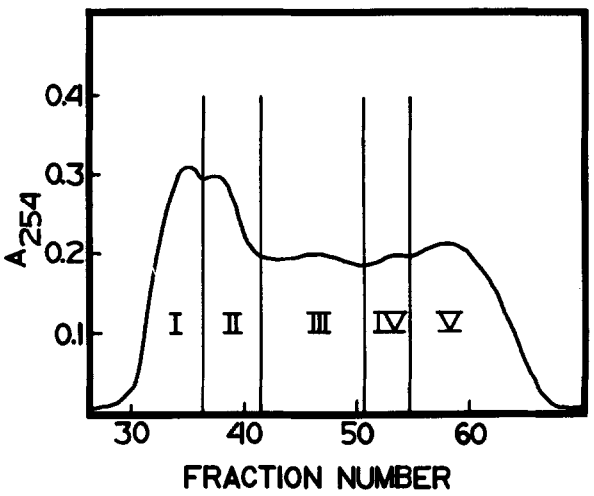
An increase in the sodium-23 line width occurred upon introduction of

ghosts. The increase in sodium line width was not exceptionally great, but treatment of the ghosts with detergents produced marked changes. Enough ghosts were added to a 0.16 M sodium chloride solution to produce a sodium line width of 20 Hz (approximately 13 mg/cc of ghost membrane). The solution was then made 0.015 M in sodium dodecyl sulfate (SDS), at which time the solution became less opaque and almost transparent. The sodium line width increased to 25.0 Hz. Another addition of the detergent to a concentration of 0.03 M increased the line width to 30.4 Hz.

The chlorine line width was also monitored at 5.868 MHz for dispersions of ghosts in 1 M sodium chloride. During the dodecyl sulfate additions, the line width was reduced from 35.6 Hz to 15.0 Hz, which is almost the 12.5 Hz of the chlorine line width in 1 M sodium chloride. This result is similar to that which we have observed for chlorine line width changes in bovine serum albumin solution titrated with sodium dodecyl sulfate.[7]

Treatment of the erythrocyte ghosts with Triton X-100, a non-ionic detergent, dissolves the ghosts just as did the dodecyl sulfate. However, Triton X-100 at similar concentrations causes no change in the sodium resonance. If, after treatment with Triton X-100, sodium dodecyl sulfate is added to the ghost suspension, the sodium line width does increase just as in the previous experiments.

Obviously, viscosity is of little importance in these experiments, as Triton X-100 solubilization caused no change in sodium line width. However, solubilization with dodecyl sulfate probably liberates protein which freely binds sodium ion. If potassium chloride is added to the SDS solubilized samples, the sodium line width markedly narrows. The sodium line width for a sample 0.16 M in sodium chloride containing SDS treated ghosts decreased from 35.7 Hz to 28.4 Hz, when the suspension was made 0.08 M in potassium chloride in addition to the 0.16 M sodium chloride. This is explained easily by assuming competition between potassium and sodium ions for the binding sites. This has been demonstrated previously by means of other techniques.[8]



Separation of erythrocyte membranes in SDS and sodium chloride on Bio-Gel P-300; fraction number versus absorbance at 254 nm. Roman numerals refer to combinations of fractions used for nuclear magnetic resonance.

Only a slight decrease in line width to 26.9 Hz was produced by increasing the potassium chloride to 0.16 M. Even at 0.3 M potassium chloride, the sodium line width is approximately 25 Hz. The effect of initially added potassium chloride is more pronounced than that produced by changes in potassium ion concentration at higher molarity.

Erythrocyte ghosts in 0.16 M sodium chloride were broken by freeze-thawing 8 times. The line width of sodium resonance was unchanged. Addition of SDS to the broken membranes caused the sodium resonance line width to increase as before. A continuous increase in line width was noted from 0.0 to 0.05 M. Addition of potassium chloride to a concentration of 0.25 M again decreased line width from 36.9 to 24.6 Hz.

The water-soluble erythrocyte fractions (see figure) were added to 0.16 M sodium chloride (25 mg/3ml). All fractions broadened the sodium resonance, although fraction V was most effective. Upon standing for 24 hours in 0.16 M sodium chloride much of the protein precipitates out.

CONCLUSIONS

The resonance line widths of sodium-23 can be used to investigate

erythrocyte membranes. Dodecyl sulfate solubilization frees sites, presumably on protein, for sodium ion binding and exchange. Potassium ions will compete for these sites.

The possibility that SDS has created sodium binding sites cannot be ruled out. However, with bovine serum albumin which binds tightly up to 10 SDS molecules, no sodium ion binding sites are formed when SDS is present at similar concentrations. Sodium ion resonances are not broadened in 0.16 M sodium chloride saturated with SDS. The water-soluble protein fractions used in the sodium ion binding study had been dialyzed extensively to remove SDS, and broadening still occurred. Whether or not all SDS had been removed is not known; however, SDS addition to these soluble fractions produced no change in sodium resonance line width.

This preliminary note indicates that the magnetic resonance technique may be of great value in studying ion interaction with proteins. We are now continuing our studies with erythrocyte and other membranes in an attempt to correlate our results with problems of ion transport.

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