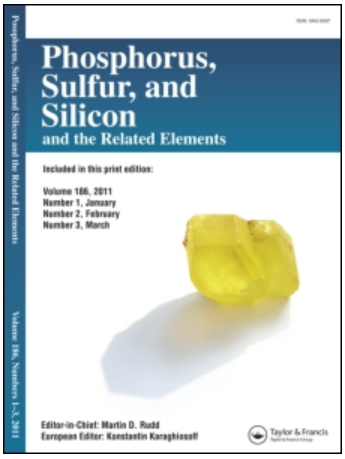

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PHOSPHORUS-31 NMR OF THE INTACT CRYSTALLINE LENS: I. THE LIVING LENS SPECTRUM. II. THE SPECTROSCOPIC EFFECTS OF DEUTERIUM OXIDE INCUBATION

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Abstract Spectra of intact bovine and canine crystalline lenses in vitro at 37° C were generated using phosphorus-31 nuclear magnetic resonance (NMR). Lens incubation in a pure D_2O buffer results in a progressive narrowing of the tissue P-31 NMR signals.

INTRODUCTION

Incubation of lenses in a pure D_2O buffer results in a time-dependent narrowing of tissue P-31 signals.[1, 2]

The Lens Phosphorus Spectrum

Figure 1 shows an intact bovine crystalline lens phosphorus-31 NMR spectrum. Chemical shift and concentration data are presented in the Table. The intralenticular pH measured from Pi is 6.8. Of special note in this lens spectrum relative to those of other species previously published is the strong Pi signal. Note that differences can be observed among the line widths exhibited by the separate signals. Compare, for example, the signals from the 6.0 δ resonance, Pi, and GPC, each of which arises from a single chemical species. The Pi signal is approximately twice as broad as that from GPC, while the 6.0 δ signal exhibits an intermediate line width. The line widths of all of these resonances are the same and narrow in the perchloric acid (PCA) extract spectrum from this same tissue.

Table I demonstrates the correspondence between the phosphorus

profiles obtained from the intact tissue and those obtained from PCA extracts. The compounds detected in the PCA extract spectrum are interpreted to be the same compounds which generate the intact tissue spectrum. The differences observed between the two sets of spectral data shown in Figure 1 merely reflect the greater signal resolution obtainable from the extract relative to that of the intact tissue.

DEUTERIUM OXIDE INCUBATION

The line-narrowing induced by D_2O has been observed with all mammalian lenses tested. When incubated in the D_2O buffer, the lens is capable of maintaining its phosphate profile and intralenticular pH for the first 4 hr at 37°C. During this time the signal-widths of the ATP resonances narrow.

Figure 2 shows two expanded spectra taken from the same lens at separate points during a D_2O buffer incubation. The region shown encompasses only the lens ATP γ and α group doublets. The first lens spectrum is essentially identical to control lens spectra;[2] the spectrum of the third time point shows ATP γ - and α-phosphate signals considerably narrowed. In the complete lens spectrum, the greatest line width changes are exhibited by ATP phosphate groups; no change is observed in the line width of lens glycerol 3-phosphorylcholine.[1] Other signals show intermediate degrees of signal narrowing.

Line widths at half height obtained by simulating the intralenticular P-31 spectrum of ATP are respectively: 0.0 hr, gamma group 18.8 $\pm$ 0.2 Hz, alpha group, 12.0 $\pm$ 0.6 Hz; 3.2 hr, 8.9 $\pm$ 0.5 Hz, and 10.3 $\pm$ 0.3 Hz.* The ATP gamma group line width changes are approximately linear with the logarithm of time, continuing until the tissue enters metabolic decline.

*Calculated from theoretical ATP NMR multiplets using the following chemical shift and coupling constant values; α, -10.59 δ; β, -19.03δ; γ, -5.49 δ; J coupling α - β, 17.09 Hz; β - γ, 13.43 Hz; P-31 frequency, 80.99 MHz.

Narrowing of the ATP resonances upon D_2O incubation is evidence for a solvent contribution to spectral line widths in intact tissue P-31 NMR spectra. The observation that ATP signals appear to be more affected than signals from other intralenticular metabolites, such as glycerol 3-phosphorylcholine, suggests that the interaction of water with ATP may be facilitated by the binding of the nucleotide to the cell's internal structure such that its rotational freedom is restricted. Since the magnesium complex of ATP is a moderately strong acid fully ionized at pH 6.8,[1] an acid-base equilibrium is probably not contributing to signal broadening in this instance. The most probable signal broadening factor is a water molecule of hydration situated close to the ATP polyphosphate chain at the gamma group position.

REFERENCES

1. J. V. Greiner, S. J. Kopp and T. Glonek, Invest. Ophthalmol. Vis. Sci, 23, 14 (1982) and references therein.
2. S. J. Kopp, T. Glonek and J. V. Greiner, Science, 215, 1622 (1983).
3. Supported by intramural resources of the Chicago College of Osteopathic Medicine and NIH grant NEI EY-03988.

TABLE I Bovine lens phosphorus-31 nuclear magnetic resonance data.

Phosphatic Compound	Amount (as % P detected) In Lens	In Extracts	Phosphatic Compound	Amount (as % P detected) In Lens	In Extracts
U		0.1	NADP 2'P		0.4
U		1.0	CP	5.9	3.1
U		0.3	PI	15.3	16.8
U	2.5	1.8	GLU 1-P		0.2
Hexose 6-P		0.6	GPE	1.7	1.5
U		0.3	GPC	7.1	6.7
U		1.5	PCr	1.1	0.9
α GP		1.7	ATP	39.6	37.3
Fru 1,6-diP	11.9	0.5	ADP	4.6	5.6
Rib 5-P		2.6	NAD	8.0	7.7
IMP		2.6	UDP-Gal		0.6
AMP		2.8	UDP-Glu	2.3	1.1
			UDP-Man		0.4

FIGURE CAPTIONS

Figure 1. Top. P-31 lens spectrum. Bottom. Lens extract spectrum.

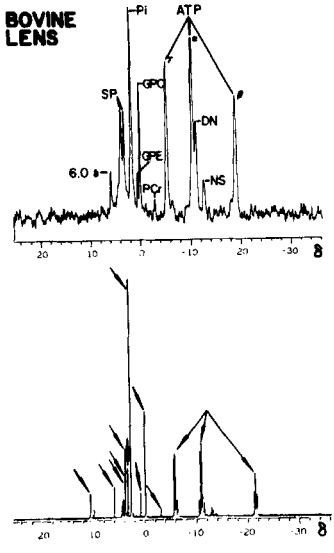

Figure 2. Lens ATP α - and γ-group doublets.