Interaction of Mn^{2+} and Gd^{3+} with Phytic Acid

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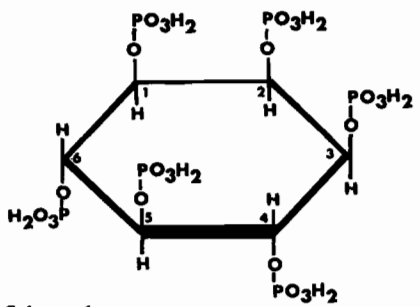
Introduction

Phytic acid (Scheme 1), the hexaphosphate of myo-inositol, is involved in the biosyntheses occurring within plant seeds and the pentaphosphate derivative functions as an allosteric effector of hemoglobin [1–3]. Furthermore, myo-inositol 1,4,5-triphosphate is involved in the intracellular mobilization of calcium, from non-mitochondrial vesicular stores, in liver cells [4].



Scheme 1.

Phytic acid is found to occur to a large extent in seeds of a variety of plants, most notably soybeans [1–3, 5, 6]. Processing of the soybean meal for human consumption requires the removal of the phytic acid because of its ability to render proteins insoluble, and in addition to sequestering important trace metal-ions needed for biological function and thereby causing a deficiency [1, 2, 5–9]. Moreover, phytic acid appears to be a large storage center for plant-seed phosphate, which is unfortunately not readily available for human use [1, 2].

Because of the biological importance of phytic acid–metal-ion interactions, we investigated the nature of Gd^{3+} and Mn^{2+} interactions with this molecule. In this report we show that Mn^{2+} preferentially interacts with the axial phosphate group at C2 of phytic acid at neutral pH. We have also found that Gd^{3+} appears to non-specifically interact with the phosphate groups of phytic acid. Moreover, at high Gd^{3+} concentrations, a Gd^{3+}–phytic acid complex appears to precipitate.

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Experimental

Materials and Methods

Myo-inositol 2-monophosphate and phytic acid (dodecasodium salt) were purchased from Sigma Chemical Company, St. Louis, Mo. Gadolinium oxide (99.9%) was purchased from Alfa Products, Danvers, Mass. and converted into the gadolinium chloride. Manganous chloride was of reagent grade quality.

^{13}C NMR spectra were recorded on a JEOL FX90Q instrument operating at 22.5 MHz (2.1 T) in the FT mode, as described previously [10]. Spin-lattice relaxation times (T_1 values) of the various carbon atoms were determined by the PRFT method [11], using 8 τ values; estimated precision was ±0.1 s. Longitudinal electron–nuclear relaxation rates $[(T_1^e)^{-1}]$ were determined for the respective carbon atoms using the equation given below [12]:

$$(T_1^e)^{-1} = (T_1^{-1})_M - (T_1^{-1})_O$$

In this equation $(T_1^{-1})_M$ and $(T_1^{-1})_O$ reflect the carbon atom relaxation rates in the presence and absence of metal-ions, respectively.

For phytic acid–Gd^{3+} and Mn^{2+} interaction studies, additions of metal-ion stock solutions to the samples were made using an Eppendorf digital pipet with the total additions ranging from 6 to 80 μl.

Results and Discussion

Phytic acid can exist in two chair conformations: one with a single phosphate group in the axial (ax) position (1 ax/5 eq) and one with a single phosphate group in the equatorial (eq) position (1 eq/5 ax) [13–15]. The predominance of a given conformation depends on such factors as pH and the counter ion present. At neutral pH and in the presence of Na^+, phytic acid exists in the 1 ax/5 eq chair conformation, with the C2 phosphate in the axial position [13–15].

The interaction of transition metal-ions with phytic acid is of immense importance because the 'chelation' of these ions by phytic acid renders them unusable for assimilation. Various structures and complexes have been proposed for the transition metal-ion–phytic acid complex; many are non-stoichiometric [9]. Therefore, in order to understand the nature of the phytic acid–metal-ion interactions we employed Mn^{2+} and Gd^{3+} and utilized electron–nuclear relaxation rates. Mn^{2+} and Gd^{3+} were employed because they are relaxation reagents [10, 12] and they also mimic Mg^{2+} and Ca^{2+} in biological systems. In these studies, only traces of Mn^{2+} or Gd^{3+} were added because a small amount of these ions causes a dramatic change in T_1^e and more

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TABLE I. ^{13}C Chemical Shift Data for Myo-inositol, Phytic Acid, and Variously Phosphorylated Myo-inositols[a]

Carbon atoms[b]	Compound			
	Myo-inositol[c]	Myo-inositol 2-monophosphate	Myo-inositol[d] 1,4,5-triphosphate	Phytic acid
1	72.1	72.5	76.3	74.8
2	73.2	78.4	72.2	75.7
3	72.1	72.5	73.0	74.8
4	73.4	74.3	75.0	77.1
5	75.3	76.0	78.4	78.8
6	73.4	74.3	71.6	77.1

[a]Given at pH ~ 6.0. [b]See structure for carbon atom numbering scheme. [c]Obtained from reference 16. [d]Obtained from reference 17. These authors used dioxane at 67.4 ppm as an internal standard. Therefore to compare their data with ours, 0.46 ppm must be added to their chemical shifts.

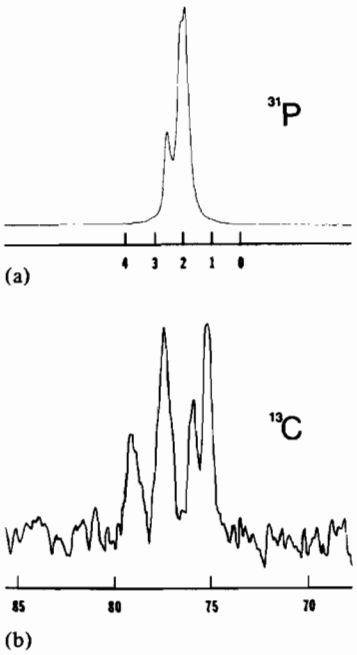

Fig. 1. Proton-decoupled, natural abundance ^{31}P and ^{13}C spectra of phytic acid. Sample concentration was 300 mM in H_2O at pH 7.0. The ^{31}P spectrum is referenced downfield with respect to external 85% H_3PO_4. The ^{13}C spectrum is referenced downfield relative to external Me_4Si. The upper trace required 570 accumulations as opposed to 4072 in the lower trace.

importantly, a very large addition of any metal-ion causes a precipitation of the sample.

Figures 1a and 1b show the proton-decoupled, natural abundance ^{31}P and ^{13}C NMR spectra of phytic acid at neutral pH. Due to the increased resolution in the ^{13}C NMR spectrum, the use of this nucleus is more advantageous for the investigation of Gd^{3+} and Mn^{2+} interactions with phytic acid. The ^{13}C resonance assignments for phytic acid are given in Table I. These assignments were based on pH

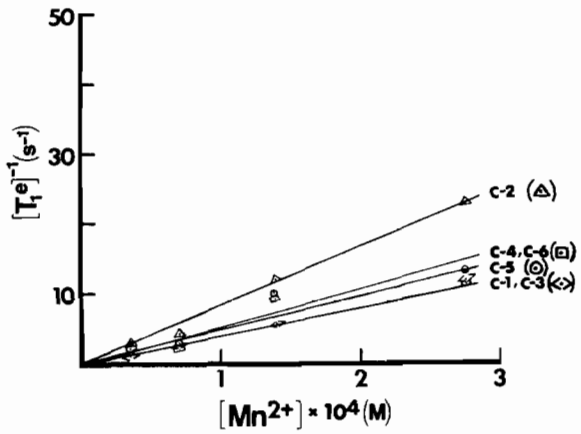

Fig. 2. The effects of added Mn^{2+} on the ^{13}C T_1^e values of phytic acid. The concentration of phytic acid was 300 mM, in H_2O at pH 7.3.

titration parameters we obtained for phytic acid and on the comparison of the ^{13}C chemical shift data of phytic acid to the ^{13}C chemical shift data of related compounds (see Table I).

Figure 2 shows a plot of $(T_1^e)^{-1}$s for phytic acid *versus* added Mn^{2+}. The substantial increase in the slope for C2 of phytic acid relative to the other carbon atoms indicates a preferential relaxation rate for this carbon atom. The enhanced relaxation probably results from the preferential binding of Mn^{2+} to the axial C2 phosphate. In the case of Gd^{3+} no preferential interaction with a particular phosphate group of phytic acid could be observed; this study also caused some problems because at higher concentrations of Gd^{3+} noticeable precipitation of the sample was observed.

In conclusion, our electron–nuclear relaxation rate studies of phytic acid showed that phytic acid does appear to contain a unique binding site for Mn^{2+}, but probably not for Gd^{3+}.

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