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Note

The phosphorylation of *epi*-inositol and *muco*-inositol with polyphosphoric acid

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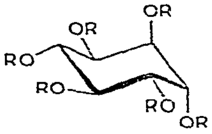
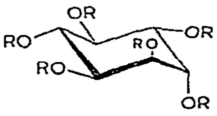
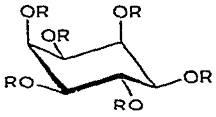
Identification of the pentakisphosphates produced by the action of a bacterial (*Pseudomonas* sp.) phytase on *myo*-inositol hexakisphosphate, D-*chiro*-inositol hexakisphosphate (2), and L-*chiro*-inositol hexakisphosphate (4) made it possible to suggest a simple model for the active centre of this enzyme[1]. In order to extend the scope of these studies, the hexakisphosphates of other inositols were required. Accordingly, the preparation of *epi*-inositol hexakisphosphate (6) and *muco*-inositol hexakisphosphate (8) was attempted.



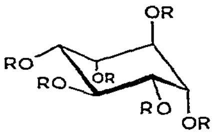
1 R = H
2 R = PO_3H_2



3 R = H
4 R = PO_3H_2



5 R = H
6 R = PO_3H_2



7 R = H
8 R = PO_3H_2

The phosphorylation of *epi*-inositol (5) with polyphosphoric acid[2] (PPA) followed by fractionation of the products by ion-exchange chromatography gave, as the main product, a phosphate whose properties were consistent with those of a tetrakisphosphate rather than a hexakisphosphate (Fig. 1). Small amounts of higher esters were present but were shown to be *myo*-inositol esters; a trace of *myo*-inositol was present as an impurity in the *epi*-inositol used for this preparation. The phosphorus–inositol ratio (4.1:1) of the main product confirmed its identity as a tetrakisphosphate. When the barium salt of the tetrakisphosphate was converted into the free acid and again treated with PPA, examination of the products by ion-exchange chromatography showed that phosphorylation beyond the tetra-stage had still not taken place.

The phosphorylation method used for *epi*-inositol[2] requires relatively large amounts of inositol. For the phosphorylation of *muco*-inositol (7), therefore, a modified procedure was devised which gave improved yields and could be used on as little as 10 mg of inositol. The main product had a phosphorus–inositol ratio of 5.89:1

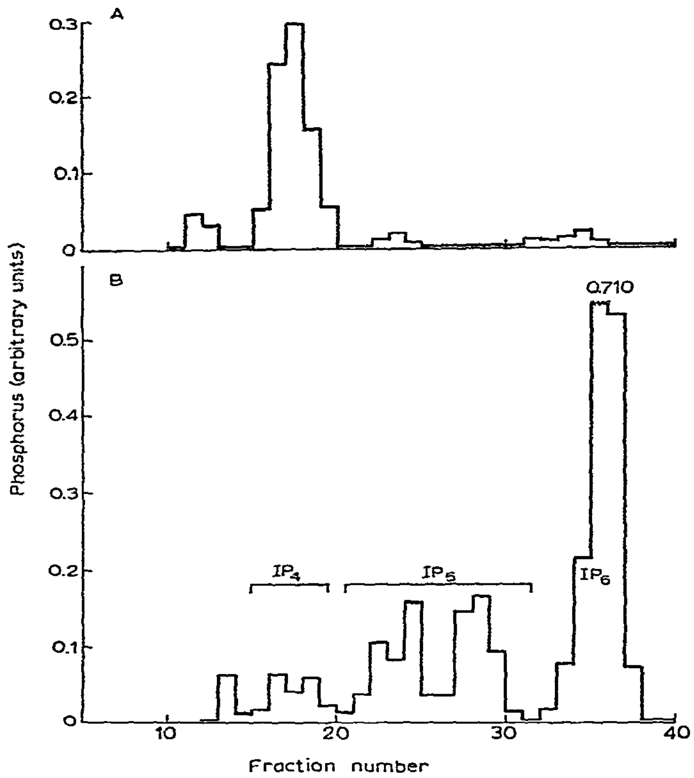
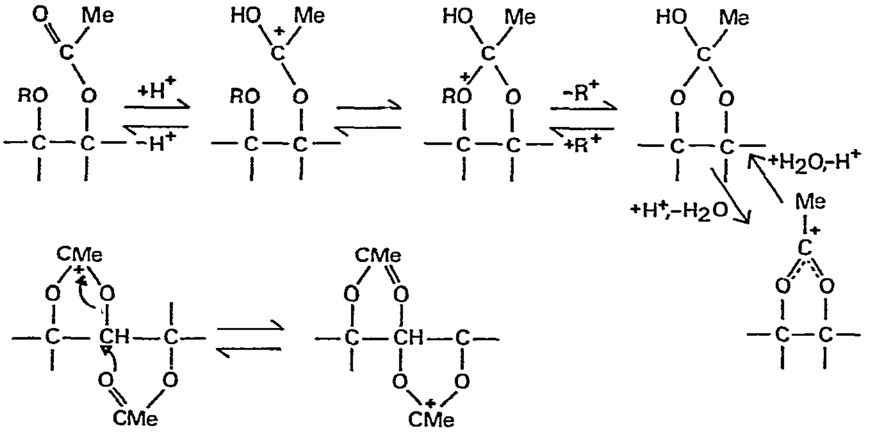


Fig. 1. Elution pattern (0.0–1.0M HCl gradient, 50-ml fractions) from a Dowex AG-1 (x2; Cl$^-$ form; 200–400 mesh) resin column of (A) phosphorylated *epi*-inositol (Ba salt; 70 mg), (B) phosphorylated *myo*-inositol (Ba salt; 70 mg). IP$_4$, *myo*-inositol tetrakisphosphates; IP$_5$, *myo*-inositol pentakisphosphates; IP$_6$, *myo*-inositol hexakisphosphate.

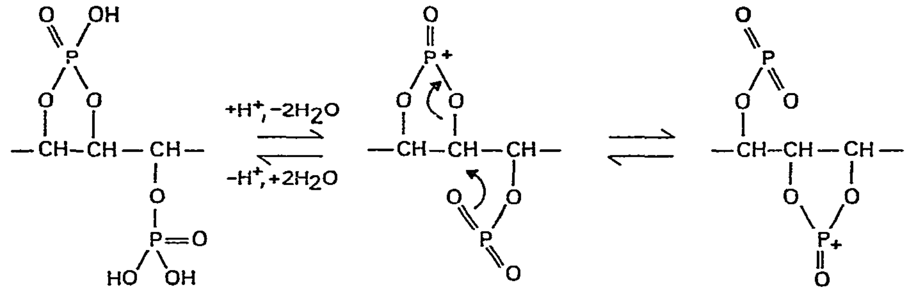
(*myo*-inositol hexaphosphate by the same method gave 5.91:1) and is therefore the hexakisphosphate. In an attempt to improve the yield, phosphorylation was continued for 24 h instead of 6 h. The yield was not appreciably increased, and examination of the product showed that a proportion (15%) of it had been epimerized to *chiro*-inositol hexakisphosphate (**2**+**4**) (Fig. 2); no *chiro*-inositol hexakisphosphate could be detected in products from a 6-hour phosphorylation of *muco*-inositol.

Epimerization of inositols by heating in PPA has not been reported. Products from a 24-hour phosphorylation of *myo*-inositol were found to contain only *myo*-inositol; D-*chiro*-inositol (**1**) behaved similarly. It is well known, however, that epimerization occurs at the middle carbon atom of each *cis,trans* sequence of hydroxyl groups when inositols are heated in acetic acid solution with a strong acid[3]. Under such conditions, *myo*-inositol is converted into an equilibrium mixture of *myo*-inositol (54%), *chiro*-inositol (41%), and *muco*-inositol (5%), a result in keeping with the low stability of *muco*-inositol relative to the other two[3]. The suggested mechanism[3] is depicted in Scheme 1.



Scheme 1

Under the strongly acidic and dehydrating conditions of the reaction of cyclitols with PPA, epimerization is probably facilitated through the formation of cyclic esters and metaphosphate esters in the manner shown in Scheme 2.



Scheme 2

By analogy with the acetates, the equilibrium would be in favour of the transformation of *muco*-inositol phosphates to *chiro*-inositol phosphates.

An explanation of the inability of PPA to phosphorylate *epi*-inositol beyond the tetrakisphosphate stage can be arrived at by consideration of the preferred conformation of this cyclitol (5). Phosphorylation of the axial groups may be difficult, owing to the great increase in non-bonded strain ("1,3-*cis* interaction") associated with the introduction of such bulky substituents. Similar non-bonded strain is also apparent in the chair conformation of *muco*-inositol, but Brownstein[4] has shown that, in this cyclitol, rapid chair–chair inversion takes place, probably through intermediate boat-forms. Thus, no two axial groups are permanently in a 1,3-*cis* situation.

An attempt to prepare a crystalline dodeca-sodium salt of *muco*-inositol hexakisphosphate by the method of Johnson and Tate[5] was not successful, although the corresponding, crystalline sodium salts of *scyllo*-inositol hexakisphosphate and *neo*-inositol hexakisphosphate have been prepared by this method[6]. Attempts to prepare crystalline dodeca-sodium salts of D-*chiro*-inositol hexakisphosphate and L-*chiro*-inositol hexakisphosphate have been unsuccessful[6].

EXPERIMENTAL

Complete descriptions of the methods used for large-scale phosphorylation of inositols, the ion-exchange chromatographic fractionation of the products, and identification and isolation of components have been published[2,7,8].

Inositols. — *epi*-Inositol (5) was prepared from *myo*-inositol by a standard procedure[9]. *muco*-Inositol (7) was prepared by the method of Suami, Lichtenthaler, and Ogawa[10], and purified by the method of Mills and Angyal[11]. A solution of *muco*-inositol (0.5 g) in water (5 ml) was run on to a column (40 x 2.5 cm) of Dowex AG-1 resin (x2; HO^- form; 200–400 mesh). The column was washed with water, and fractions of eluate (50 ml) were evaporated to small volume and analysed by chromatography (Whatman No. 1 paper, 85% aqueous acetone). Visualization of the inositol zones[12] enabled the *muco*-inositol-containing fractions (21–36) to be identified. These were combined and evaporated to dryness, and the residue was crystallized from aqueous alcohol.

Phosphorylation of muco-*inositol.* — Finely powdered *muco*-inositol (0.05 g) was dissolved in warm (60°) orthophosphoric acid (85%; 0.1 ml). To this solution was added PPA (P_2O_5 82–85%, 2 g), and the resulting mixture was heated in a sealed tube (150°; 6 or 24 h). The product was cooled, mixed with water (25 ml), and then heated (100°) for 3 h to decompose all inorganic polyphosphates to orthophosphate[13]. The solution was diluted to 500 ml with water and passed through a column (17 x 1.0 cm) of Dowex AG-1 resin (x2; Cl^- form; 200–400 mesh). The column was then washed with 0.2M HCl (1000 ml) and finally eluted with a gradient of HCl (0–1M). A result qualitatively similar to that shown in Fig. 1 for the fractionation of the *myo*-inositol phosphorylation products was obtained. The main phosphorus-containing component (Fractions 30–34) was recovered as a barium salt (0.168 g, 41%, 6 h; 0.180 g, 44%, 24 h).

Determination of phosphorus–inositol ratios. — Barium salts of inositol phosphates were converted into the free acids and hydrolysed[8] in 6M HCl (110°, 40 h). HCl was removed from the hydrolysate by vacuum evaporation, and the phosphorus content of the residue was determined by a modified Dickman and Bray method[7]. Where a sufficiently large sample was available, *i.e.*, for *epi*-inositol tetrakisphosphate, inositol was assayed by the Launer[14–16] method; otherwise, the Lornitzo[17] method was used.

Identification of chiro-*inositol hexakisphosphate.* — The minor component present in the 24-h phosphorylation products of *muco*-inositol was identified as *chiro*-inositol hexakisphosphate by co-chromatography with a known sample[2] (Fig. 2). The free inositol released by hydrolysis[7] of this component was identified[7] as *chiro*-inositol by paper chromatography in aqueous acetone (85%).

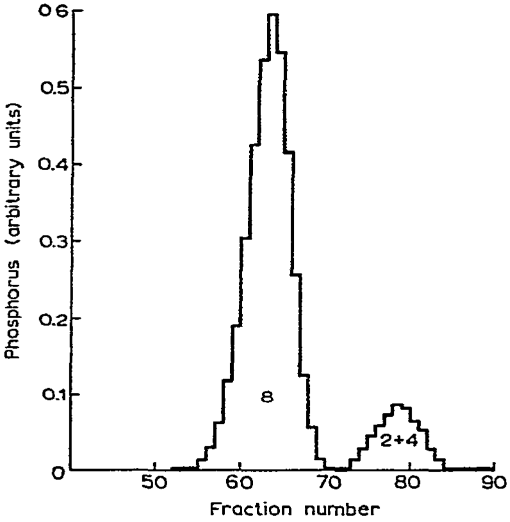


Fig. 2. Elution pattern (0.61M HCl, 10-ml fractions) from a column (61 × 1.0 cm) of Dowex AG-1 (x2; Cl$^-$ form; 200–400 mesh) resin of *muco*-inositol hexakisphosphate (24-h phosphorylation; Ba salt; 15 mg). *muco*-Inositol hexakisphosphate (8) (85%), *chiro*-inositol hexakisphosphate (2+4) (15%).

ACKNOWLEDGMENTS

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