# Mechanism of the *myo*-Inositol Phosphatase Reaction†

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*myo*-Inositol phosphatase from bovine brain operates *via* a ping-pong mechanism whereby phosphate is transferred from the substrate to the enzyme and then from the enzyme to water; lithium cation inhibits the second step.

Mammalian brain inositol phosphatase[1,2] catalyses the hydrolysis of both enantiomers of *myo*-inositol-1-phosphate[3] and *myo*-inositol-4-phosphate.[4] The enzymes from bovine brain[1,5] and rat brain[6] are inhibited by lithium cation in an uncompetitive manner as was determined by the observation that increasing concentrations of $Li^+$ give parallel line double reciprocal plots with intersects at corresponding lower substrate $K_M$ and $V_{max}$ values. On the other hand, inhibition by $Li^+$ is non-competitive with respect to the essential divalent metal ion $Mg^{2+}$ (apparent $V_{max}$ decreases, $K_M$ for $Mg^{2+}$ is unaltered). The inhibition of the enzyme by $Ca^{2+}$ is competi-

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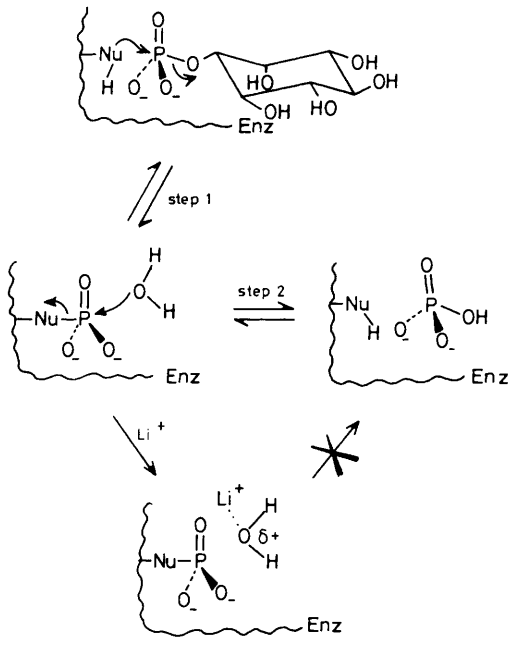
tive for $Mg^{2+}$ and thus it appears that $Li^+$ does not bind at the $Mg^{2+}$ site or affect $Mg^{2+}$ binding.

Uncompetitive inhibition is rare[7] and in view of the now well established intermediacy of phosphorylated enzyme species[8,9] generated during the catalytic cycle of phosphatases and phosphoryl transferases in general, the possibility of the occurrence of a ping-pong type phosphoryl transfer mechanism (Scheme 1) was investigated.

Preliminary experiments designed to trap the phosphoryl group failed. Endogenous inositol added to assay mixtures at up to 50 mM had no effect upon the reaction rate. Enzyme rate assays in which various concentrations of the alternative nucleophile hydrogen sulphide were added showed only minor deviation from the standard rates and only at very high concentrations. It was noted with interest however, that the inhibitory effect of $Li^+$ decreased with increasing pH, whereas the competitive inhibitory effect of phosphate increased with increasing pH. It was also noted that values of $V_{max}$ for a variety of phosphate monoesters did not differ widely.[2]

$$RO\text{-}PO_3^{2-} + E \underset{-ROH}{\overset{\text{Step 1}}{\rightleftharpoons}} E\text{-}PO_3^{2-} \underset{+H_2O}{\overset{\text{Step 2}}{\rightleftharpoons}} E + HOPO_3^{2-}$$

**Scheme 1**



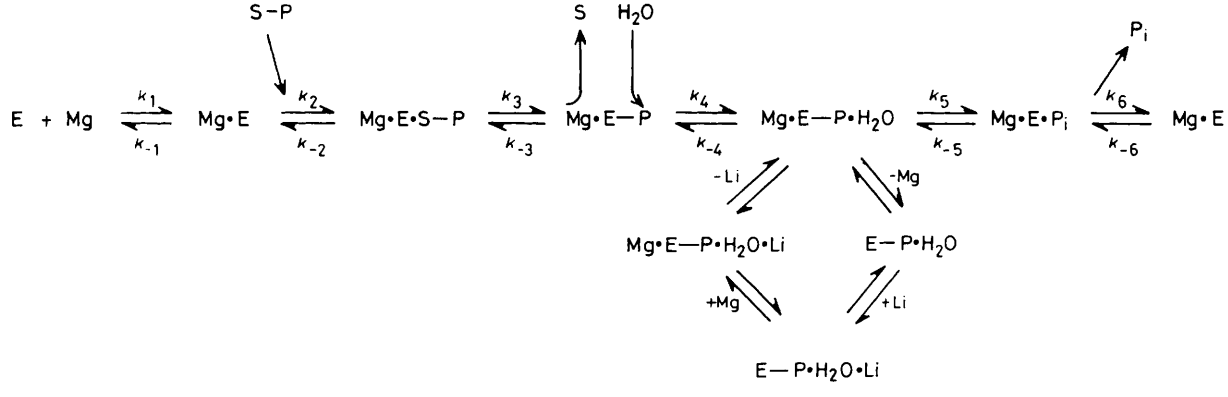

**Scheme 2**

In view of our inability to replace the phosphoryl acceptor molecule, water, by other nucleophiles and further detect the change kinetically, we followed an alternative approach. It was reasoned that if the reaction occurred *via* a two step ping-pong mechanism then $Li^+$ could inhibit the reaction either before hydrolysis of the phosphate ester (step 1, Scheme 1) or before transfer of the phosphoryl group to water (step 2, Scheme 1). Since phosphate is a competitive inhibitor for the substrate[1] and since inositol has no effect, it was expected that any products formed during the $Li^+$ inhibited reaction would be released from the enzyme.

Accordingly, high specificity activity [$^{14}$C]inositol-1-phosphate was incubated with $Li^+$ saturated enzyme and the release of [$^{14}$C]inositol was measured at regular intervals over a period of 30 min against a control containing no enzyme. Analysis of the results revealed that after an initial rapid release of [$^{14}$C]inositol (all detected in the first measurement) no further reaction occurred. Further experiments revealed that the amount of [$^{14}$C]inositol released was directly proportional to the activity of the enzyme used in the incubation.

From these observations it is apparent that the catalysed reaction indeed occurs *via* a ping-pong mechanism and that $Li^+$ inhibition occurs after phosphate ester hydrolysis. In view of the fact that phosphate is a competitive inhibitor for the substrate[1] and if formed should dissociate to allow a new substrate molecule to occupy the active-site, the results also indicate that $Li^+$ exerts its effect before the dephosphorylation of the enzyme and the formation of inorganic phosphate.

The mechanistic interpretation above is entirely consistent with, and also rationalizes, the apparent uncompetitive mode of $Li^+$ inhibition with respect to the substrate. It is well established that ping-pong reactions give characteristic parallel line double reciprocal plots when the concentration of the second substrate is altered. As the concentration is increased, $V_{max}$ and the $K_M$ of the first substrate increase.[10] In our analysis, the parallel line reciprocal plots indicative of uncompetitive inhibition, where increasing $Li^+$ leads to a decreasing $V_{max}$ and $K_M$ for the phosphate ester, should be interpreted as decreasing availability of active-site water. That is, the effective concentration of water available to dephosphorylate the phosphoryl enzyme intermediate is reduced as $Li^+$ concentration increases. Indeed, it seems quite reasonable that $Li^+$ could exert its effect, either by co-ordinating to active site water molecules in a manner which reduces their nucleophilicity and/or mobility; or, by altering the geometry of the active site to a form less able to effect dephosphorylation (Scheme 2).




**Scheme 3**

The observed non-competitive inhibition of the enzyme against the essential divalent cation $Mg^{2+}$ by $Li^+$ ($K_M$ for $Mg^{2+}$ is unaltered, $V_{max}$ decreases with increasing $Li^+$) fits well into this mechanistic scheme. Given that $Li^+$ only inhibits the dephosphorylation reaction and binds at an otherwise unoccupied site, then it is reasonable to expect catalytically active $Mg^{2+}$·enzyme·substrate complexes and the inhibited $Li^+ \cdot Mg^{2+}$ phosphoryl enzyme complex ($Mg \cdot E\text{–}P \cdot H_2O \cdot Li$) to share identical dissociation constants for the loss of $Mg^{2+}$ (Scheme 3).

This mechanism predicts that the apparent value of the $K_i$ for $Li^+$ should be substrate dependent, as is observed.[1,2]

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