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

Non-competitive 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]

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.

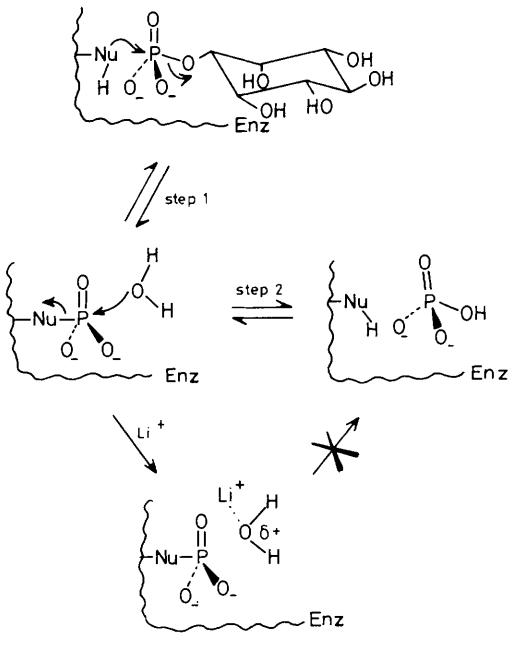
Accordingly, high specificity activity [^{14}C]inositol-1-phosphate was incubated with Li^+ saturated enzyme and the release of [^{14}C]inositol was measured at regular intervals over a period of 30 min against a control containing no enzyme. Analysis of the results revealed that after an initial rapid 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.

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

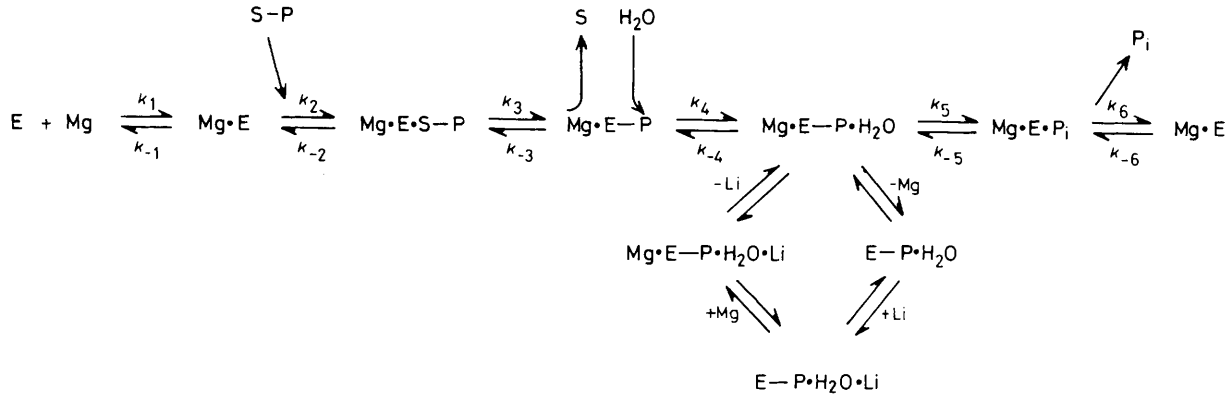
Scheme 1

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 non-competitive 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 non-competitive 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 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^+·Mg^{2+} phosphoryl enzyme complex (Mg·E–P·H_2O·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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References

1 N. S. Gee, C. I. Ragan, K. J. Watling, S. Aspley, R. G. Jackson, G. G. Reid, D. Gani, and J. K. Shute, *Biochem. J.*, 1988, **249**, 883.
2 J. K. Shute and D. Gani, unpublished results.
3 D. C. Billington, R. Baker, J. J. Kulagowski, and I. M. Mawer, *J. Chem. Soc., Chem. Commun.*, 1987, 314.
4 D. C. Billington and R. Baker, *J. Chem. Soc., Chem. Commun.*, 1987, 1011.
5 L. M. Hallcher and W. R. Sherman, *J. Biol. Chem.*, 1980, **255**, 10896.
6 K. Takimoto, M. Okada, Y. Matsuda, and H. Nakagawa, *J. Biochem.*, 1985, **98**, 363.
7 A. Cornish-Bowden, *FEBS Lett.*, 1986, **203**, 3.
8 J. R. Knowles, *Annu. Rev. Biochem.*, 1980, **49**, 877.
9 H. G. Floss, M. Tsai, and R. W. Woodward, *Top. Stereochem.*, 1984, **15**, 253.
10 A. Fersht, 'Enzyme Structure and Mechanism,' Freeman, New York, 1985, 2nd edn., ch. 3, p. 98.