BIOCHEMICAL LETTERS

Inhibition of inositol 1,4,5-trisphosphate 3-kinase by heparin: basal and Ca^{2+} /calmodulin-stimulated activity

Inositol 1,4,5-trisphosphate (InsP_3) 3-kinase is responsible for the synthesis of InsP_4 . The kinase in many tissues is regulated by Ca^{2+} /calmodulin (CaM; see [1] for review). The activity of purified enzyme from bovine brain is stimulated up to 30-fold by Ca^{2+} /CaM. Stimulation by CaM appears to result from an increase in enzyme maximal velocity without a substantial change of K_m for InsP_3 [2].

Similar changes in kinetics have been reported for another well-characterized CaM-regulated enzyme, the cyclic nucleotide CaM-sensitive phosphodiesterase [3]. Increases of activity of that enzyme are also 20–40-fold when assayed in the presence of Ca^{2+} and saturated CaM as compared to basal activity assayed in the presence of EGTA. Thus activity of both enzymes could be measured in basal and Ca^{2+} /CaM-stimulated assay conditions. In this context, we have previously reported that phosphodiesterase competitive inhibitors (e.g. methylxanthines) are more potent on the Ca^{2+} /CaM-stimulated enzyme than on the basal phosphodiesterase activity [4]. We thus expected to observe quite different sensitivity of InsP_3 to kinase inhibitors when assayed in basal and Ca^{2+} /CaM-stimulated conditions.

In a screening of affinity columns, InsP_3 3-kinase could be adsorbed onto heparin–Sephadex (Pharmacia) and eluted in buffer containing high salt (1 M-NaCl) or 0.5 mM- CaCl_2 /0.1 μM -CaM. Moreover, heparin (Sigma H-3125), previously identified as a competitor of InsP_3 binding to its receptor [5], inhibited InsP_3 3-kinase activity, affecting both $V_{\max}$ and K_m for InsP_3 (mixed-type inhibition). Basal activity (assayed at 1 mM-EGTA) was strongly inhibited with an IC_{50} (concentration producing 50% inhibition) of 0.5 $\mu\text{g}/\text{ml}$ as compared to stimulated activity (IC_{50} 10 $\mu\text{g}/\text{ml}$) at 1 μM - InsP_3 . Our results have a practical implication: the sensitivity of InsP_3 3-kinase to any potential inhibitor has to be studied on basal and Ca^{2+} /CaM-stimulated activity before comparing the data with other InsP_3 metabolic enzymes or receptors. In the InsP_3 receptor studies of Worley *et al.* [5], half-maximal inhibition of InsP_3 binding occurred with heparin at 5 $\mu\text{g}/\text{ml}$.

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- Irvine, R. F., Moor, R. M., Pollock, W. K., Smith, P. M. & Wreggett, K. A. (1988) *Philos. Trans. R. Soc. London Ser. B* **320**, 281–298
- Takazawa, K., Passareiro, H., Dumont, J. E. & Erneux, C. (1989) *Biochem. J.* **261**, 483–488

- Kincaid, R. L. & Vaughan, M. (1988) *Methods Enzymol.* **139**, 557–573
- Miot, F., Dumont, J. E. & Erneux, C. (1983) *FEBS Lett.* **151**, 273–276
- Worley, P. F., Baraban, J. M., Supattapone, S., Wilson, V. S. & Snyder, S. H. (1987) *J. Biol. Chem.* **262**, 12132–12136

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Some comments on the type references of the official nomenclature (IUB) for β -N-acetylglucosaminidase, β -N-acetylhexosaminidase and β -N-acetylgalactosaminidase

Although intensive studies have been carried out on the characterization of these three glycosidases isolated from very different materials, there is still much to know about their peculiarities, regulation and physiological significance in the catabolism of glycoconjugates (glycoproteins, glycolipids and glycosaminoglycans).

β -N-Acetylhexosaminidase, also known as *N*-acetyl- β -hexosaminidase or hexosaminidase (EC 3.2.1.52), which has been studied in our Department from some species of molluscs (Pérez & Cabezas, 1977; Calvo *et al.*, 1978; Villar *et al.*, 1984) has a higher activity on natural substrates than that from some vertebrates (Rodríguez-Hernández *et al.*, 1987; Sánchez-Bernal *et al.*, 1988) although the enzyme from both sources shows a significant activity towards *p*-nitrophenyl glycosides. β -N-Acetylhexosaminidase from all the above-mentioned sources has a dual activity: β -N-acetylglucosaminidase and β -N-acetylgalactosaminidase. The ratio of the two activities remains unaltered through the entire purification procedure, this ratio being between 2.5 and 4 for the mollusc enzyme and 10 for that of pig brain (J. García-Alonso, A. Reglero & J. A. Cabezas, unpublished work). Moreover, the same thermal inactivation, pI and behaviour towards mixed substrates, as well as very similar pH optima and K_i values for competitive inhibitors, suggest that in these organisms at least β -N-acetylglucosaminidase and β -N-acetylgalactosaminidase are catalysed by a single protein and at the same active site (see the above-mentioned papers).

A problem arises when authors wish to use the correct EC number and the exact name of the official recommendations for enzyme nomenclature [IUB (International Union of Biochemistry), 1984] to report their results. In fact, two EC numbers and two names (EC 3.2.1.30 for β -N-acetylglucosaminidase and EC 3.2.1.52 for β -N-acetylhexosaminidase) can probably be employed for designating the same activities of the enzyme(s), according to the latest recommendations (IUB, 1984).