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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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).

Theoretically, one might be assisted by consulting the type references corresponding to each enzyme. Thus, five type references for β -*N*-acetylglucosaminidase are in the present edition of Enzyme Nomenclature (1984), exactly the same references as those of the previous editions of 1978 (IUB, 1978) and 1972 (IUB, 1972). The most recent of these references is that of Li & Li (1970); however, this one and those of Findlay & Levvy (1960) and Woollen *et al.* (1961) indicate the dual activity of the enzyme, unlike the other two references (Zechmeister & Toth, 1939; Pugh *et al.*, 1957) which correspond to old and probably obsolete papers published at a time when this dual activity had not been discovered. The latest recommendations (IUB, 1984) accept that "various examples of the enzyme act on *N*-acetyl- β -D-galactosaminides and chitobiose", in addition to what is considered as the main activity, i.e. the "hydrolysis of terminal non-reducing *N*-acetyl- β -D-glucosamine residues in chitobioses and in higher analogs and in glycoproteins". So, one might deduce that the 'various examples' of the former sentence are those supported by the references of Li & Li (1970), Findlay & Levvy (1960) and Woollen *et al.* (1961).

On the other hand, β -*N*-acetylhexosaminidase (EC 3.2.1.52) was a designation officially incorporated for the first time as late as 1972 (IUB, 1972), since it was not included either in the 1961 (IUB, 1961) or in the 1964 (IUB, 1964) editions of these reports or recommendations, while β -*N*-acetylglucosaminidase (EC 3.2.1.30) was.

β -*N*-Acetylhexosaminidase (EC 3.2.1.52) was initially supported in the 1972 edition of Enzyme Nomenclature only by the reference corresponding to the paper of Frohwein & Gatt (1967); in the edition of 1978, by this reference and that of Li & Li (1970) (which also supports EC 3.2.1.30), and, in the latest edition of 1984, by those two references and that of Calvo *et al.* (1978).

Thus, it seems that β -*N*-acetylhexosaminidase (EC 3.2.1.52) is a well-documented enzyme, supported by consolidated studies which demonstrate the presence of (at least) two activities catalysed by a single protein, which has been well characterized. In contrast, one might doubt if the designation β -*N*-acetylglucosaminidase (EC 3.2.1.30), virtually equivalent to that of β -*N*-acetyl-galactosaminidase (EC 3.2.1.52), should be employed in a general manner, as is frequently done (see below).

Another related enzyme, β -*N*-acetylgalactosaminidase (EC 3.2.1.53) was included for the first time in the nomenclature recommendations of 1972, with only one type reference (Frohwein & Gatt, 1967). In the editions of 1978 and 1984, this enzyme has maintained this sole type reference.

One question arises: does a true β -*N*-acetylgalactosaminidase activity associated with a single protein exist and is it well characterized at least from one source? According to Frohwein & Gatt (1967), this activity is predominant in a fraction separated from calf brain, where it has been characterized.

There is another question concerning the two activities: is it possible to obtain a complete separation and a detailed characterization of a single protein with β -*N*-acetylglucosaminidase activity only (i.e. deprived of β -*N*-acetylgalactosaminidase activity)? The article by Hoogwinkel *et al.* (1972) could partly answer this question. In fact, it states that there are two fractions, separated by cellulose paper electrophoresis, "one exerting *N*-acetyl-galactosaminidase activity and the other, which is only

present in brain tissue, and absent in liver tissue, shows only acetylglucosaminidase activity". However, it seems that a detailed characterization is not known.

In summary, from all these comments, one may conclude the following.

1. β -*N*-Acetylhexosaminidase (EC 3.2.1.52) is a well-documented enzyme; it possesses at least two catalytic activities, β -*N*-acetylglucosaminidase and β -*N*-acetyl-galactosaminidase, the latter being lower than the former.

2. β -*N*-Acetylglucosaminidase or *N*-acetyl- β -glucosaminidase (EC 3.2.1.30) does not seem to be well supported in the official recommendations (IUB, 1984), since two of its type references are probably obsolete, and the other three would correspond rather to β -*N*-acetylhexosaminidase (EC 3.2.1.52). Thus, it may be suggested that the official designation should be either (i) maintained only if it is confirmed by a new reference(s) (corresponding to work in which not only the occurrence but also the characterization of a sole activity associated with a single protein is demonstrated), or (ii) deleted.

3. With respect to β -*N*-acetylgalactosaminidase (EC 3.2.1.53) it seems convenient to maintain it in the official list of recommendations, supported not only by the type reference of Frohwein & Gatt (1967), as it is now, but perhaps also by Hoogwinkel *et al.* (1972), even if the latter does not report a true characterization of the enzyme.

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