Note

Desulfation of Tyrosine-O-sulfated Peptides by Some Eukaryotic Sulfatases

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Three mammalian and eight non-mammalian arylsulfatas were investigated for their activities toward tyrosine-O-sulfate (TyrS) in peptides. None of the mammalian arylsulfatas exhibited detectable activities toward TyrS-containing peptides. Of the non-mammalian arylsulfatas tested, Types VII, VIII, and H-1, 2, and 5 displayed strong activity on endo-TyrS residues. The prokaryotic sulfatase, Type VI, was active only on free TyrS and N-terminal TyrS of Leu-enkephalin. All the sulfatas were active on p-nitrophenyl sulfate and p-nitroceloate sulfate.

Key words: arylsulfatase; tyrosine sulfation; post-translational modifications

Post-translational tyrosine sulfation has a widespread occurrence among proteins of all multicellular eukaryotic organisms. It is involved with the biological roles of some of those sulfated peptides and proteins, and in their secretion from the cells. However, in some cases the functional relevance of sulfation is still unclear. Sulfation and desulfation have long been suggested to be important in the metabolic regulation of hormones, etc. (e.g., estrogen), and it is postulated that insight into the metabolic fate of protein-bound tyrosine-O-sulfate (TyrS) may throw light on this. Desulfation of tyrosine-O-sulfated peptides and proteins in vivo as a regulatory mechanism in eukaryotic cells is yet to be demonstrated. For this purpose it is necessary to establish and characterize the ability of eukaryotic sulfatases to desulfo TyrS and tyrosine-O-sulfated peptides. This paper discusses the activities of three mammalian and eight non-mammalian, i.e., seven non-mammalian-eukaryotic and one prokaryotic, arylsulfatas with respect to their abilities to hydrolyze free and peptide-bound TyrS. Their activities are also compared with those on p-nitrophenyl sulfate (PNPS) and p-nitroceloate sulfate (PNCS).

The seven non-mammalian eukaryotic arylsulfatas used in the study, i.e., Types IV and V from Patella vulgata, Types VII and VIII from Abalone entrailets, and Types H-1, H-2, and H-5 from Helix pomatia, and the prokaryotic enzyme, i.e., Types VI from Aerobacter aerogenes, were purchased from Sigma Chemical Co., U.S.A. The mammalian arylsulfatas A and B (ASA and ABS) were prepared from bovine liver and human leuocytes based on the method of Wojcjak, and the mammalian arylsulfatase C (ASC) was purified from human placenta and bovine liver according to the method of Kawano et al.

For the enzyme assays, the commercial preparations were reconstituted as prescribed by the manufacturer. The reactions with PNPS or PNCS, done in 100 µl of a suitable buffer (see the Table) containing 2.5 mM substrate, were started by the addition of 10 µl of the enzyme, allowed to proceed for 1 h at 37°C and stopped by the addition of 5 ml of 1 N NaOH. UV absorbance of the mixtures were read at 515 nm. The assays with TyrS (synthesized by the method of Jevons), cholecystokinin octapeptide (CCK-8), leucine enkephalin (Leu-enk), N-acetyl leucine enkephalin (N-acetyl-Leu-enk), and hirudin were done in the same way as above, except that in these cases, the substrates were incorporated at 0.1 mM concentration, and the reaction mixtures were analyzed by ion-pair high performance liquid chromatography (HPLC) for TyrS, or by reverse phase HPLC for other peptides. The buffer systems used for assaying the mammalian arylsulfatas ASA ASB and ASC were 0.1 M sodium acetate buffer (pH 5.0) and 0.1 M imidazole-HCl buffer (pH 7.0), respectively.

For the bovine fibrinopeptide B (Fib-P) assay, Fib-P was prepared by clotting 50 µl of 0.5% bovine fibrinogen (Nakarai Tesque, Japan) in 50 mM ammonium bicarbonate buffer with thrombin (1 NIH unit/1 mg fibrinogen). After clotting, the mixture was filtered through a Millipore UFC3TG00 filter. The filtrate was lyophilized and resolubilized in 90 µl of 0.1 M sodium acetate buffer (pH 5.0). Reactions were done by the addition of 10 µl arylsulfatase solutions, proceeded as described above, and were analysed by HPLC. To prepare N-acetyl-Leu-enk, Leu-enk was reacted with 95% acetic anhydride, and the acetylated peptide was purified by HPLC.

Protein tyrosine sulfation, a widespread modification in eukaryotes, has not been demonstrated in prokaryotes to date. However, the reversal of this modification is yet to be established in any of the animal species investigated. Arylsulfatas that catalyze the desulfation of phenolic compounds could be expected to hydrolyze TyrS in the same manner, but our results showed that desulfation of free and protein-bound TyrS by them was not similar to that of the simpler phenol-O-sulfate derivatives, such as PNCS or PNPS, and varied depending on the animal species from which they originated. Mammalian sulfatas A, B, or C showed no activity on the protein tyrosine sulfate (data not shown), although it has been reported by Dodgson et al. that ASA was active on free tyrosine sulfate. However, as shown in the Table, the non-mammalian-eukaryotic types showed varying degrees of activity on the TyrS-peptides tested. Especially those from Abalone entrailets, i.e., Types VII and VIII, displayed strong activity towards those peptides with endo-TyrS residues. These two types recorded comparatively low activity on Leu-enk with an N-terminal TyrS, but displayed a dramatic increase in activity upon N-acetylation of Leu-enk. Among the sulfatas from Helix pomatia, Types H-1 and H-5 showed similar activity, with a strong affinity for endo-TyrS residues, and a lesser activity for those on the N-terminus. However, Type H-2 from the same species displayed strong affinities for both N-terminal and endo-TyrS residues. In contrast, the prokaryotic arylsulfatase Type VI displayed no activity against CCK-8 and Fib-P, but showed a very strong

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Abbreviations: PNPS, p-nitrophenyl sulfate; PNCS, p-nitroceloate sulfate; CCK-8, cholecystokinin octapeptide; Leu-enk, Leu-enkephalin; Fib-P, fibrinopeptide; ASA, arylsulfatase A; ASB, arylsulfatase B; ASC, arylsulfatase C.
activity on Leu-enk. This activity was reduced to less than one-fifteenth when the N-terminus of the peptide was acetylated (0.215 units h mg protein). Moreover, the Table also shows that, although, all the non-mammalian sulfatases tested desulfated free TyrS. In the case of the eukaryotic enzymes, this was to a much lesser extent when compared with that of PNCS or PNPS (1:66 for Type H-2, and 1:614 for Type VII, when compared with the activity on PNCS; considered as an average for the species, these ratios were 1:175 for Patella vulgata, 1:85 for Abalone entrails, and 1:520 for Helix pomatia). In contrast, the prokaryotic enzyme, Type VI, showed almost the same activity on free TyrS as for PNCS, giving a ratio of only 1:1.2, which was at least a 400-fold activity on free TyrS at pH 7.5 when compared with the eukaryotic types at either pH 5.0 or 7.5.

The above findings clearly show that many of the non-mammalian-eukaryotic arylsulfatases tested are capable of desulfating protein-bound TyrS in a specific manner, when compared to free TyrS. On the other hand, similar activity could not be detected in the prokaryotic enzyme, in spite of it being capable of hydrolyzing free TyrS. This suggests that the eukaryotic sulfatases are designed to recognize the groups and residues flanking the TyrS in proteins, and thereby act only on conjugated TyrS. This is very similar to the situation with the sulfation of proteins, where tyrosylprotein sulfotransferase acts only on protein-bound tyrosine in specific amino acid sequences. It is therefore very likely that the sulfatases in eukaryotes have evolved to desulfate protein-bound TyrS, while the prokaryotes have not developed this characteristic since they do not possess this modification. It is very possible that during the evolutionary stages of mollusks and snails, these sulfatases have developed the ability to desulfate protein-bound TyrS, still retaining their ability to hydrolyse the simple phenyl derivatives. In mammalian tissues, however, there may have evolved specific protein-tyrosine sulfatases, which are distinct from those recognized to date (ASA, ASB, and ASC have been identified by their ability to hydrolyse PNPs and or PNCS (1-3)), and which do not act on either free TyrS or simpler phenyl derivatives. These areas need to be investigated.

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