Arylsulphatase and Glycosulphatase of *Charonia lampas* SUBSTRATE SPECIFICITY TOWARDS SUGAR SULPHATE DERIVATIVES

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The specificity of the arylsulphatase and glycosulphatase enzymes of the marine mollusc *Charonia lampas* towards a number of carbohydrate sulphate esters was examined. The results confirm that the relationship between the established nomenclature of the two enzymes and their respective specificities is inconsistent.

In the Enzyme Nomenclature recommended by IUPAC and IUB in 1972, arylsulphatase (EC 3.1.6.1), generally measured by its activity towards chromogenic substrates such as potassium *p*-nitrophenyl sulphate, was defined as the sulphohydrolase that hydrolyses aryl sulphates. On the other hand, glycosulphatase (EC 3.1.6.3), generally measured by its activity towards potassium D-glucose 6-sulphate, was classified as a sugar sulphate sulphohydrolase. These classifications are somewhat inconsistent in that some naturally occurring sugar sulphates can be hydrolysed by some arylsulphatases but not by glycosulphatase. Thus several laboratories have reported the hydrolysis of sulphogalactolipids by mammalian arylsulphatase A or by molluscan arylsulphatase (Mehl & Jatzkewitz, 1968; Jerfy & Roy, 1973; Yamato *et al.*, 1974; Fluharty *et al.*, 1974; Hatanaka *et al.*, 1975*a*, 1976*a*). The hydrolysis of UDP-*N*-acetyl-D-galactosamine 4-sulphate (Tsuiji *et al.*, 1974) is probably achieved by mammalian arylsulphatase B (Fluharty *et al.*, 1975), whereas ascorbate 2-sulphate is hydrolysed by mammalian arylsulphatase A and by molluscan arylsulphatase (Roy, 1975; Hatanaka *et al.*, 1975*b*; Fluharty *et al.*, 1976). In contrast, none of these compounds appear to be substrates for molluscan glycosulphatase (Hatanaka *et al.*, 1975*a*, 1976*a*).

The large marine gastropod *Charonia lampas* possesses a variety of different sulphatases, including glycosulphatase (Soda & Hattori, 1933), arylsulphatase (Soda & Egami, 1933), steroid sulphatase and polysaccharide sulphatases (Takahashi & Egami, 1961). Three species of sulphatase, namely glycosulphatases I and II and arylsulphatase, have previously been highly purified from the liver (hepatopancreas) of *C. lampas* and almost completely separated from each other (Hatanaka *et al.*, 1976*b*). The present paper deals with the substrate specificity of *C. lampas* sulphatases towards various sugar sulphate derivatives.

Materials and Methods

Chemicals

Potassium *p*-nitrophenyl sulphate was purchased from Boehringer Corp., Mannheim, Germany, and was recrystallized from aq. 60% ethanol. Potassium D-glucose 6-sulphate and potassium D-galactose 6-sulphate were obtained from Seikagaku Kogyo Co., Tokyo, Japan, and dipotassium 2-hydroxy-5-nitrophenyl sulphate (nitrocatechol sulphate) was from Sigma Chemical Co., St. Louis, MO, U.S.A. Potassium cortisone 21-sulphate was a gift from Dr. A. B. Roy (John Curtin School of Medical Research, Canberra, A.C.T., Australia), UDP-*N*-acetyl-D-galactosamine 4-[³⁵S]sulphate was a gift from Professor S. Suzuki (Nagoya University, Nagoya, Japan), and the potassium salts of D-galactose 4-sulphate and 6-methyl-D-galactose 4-sulphate were kindly provided by Dr. J. R. Turvey (University College of North Wales, Bangor, Gwynedd, U.K.). Potassium L-ascorbate 2-sulphate was synthesized as described by Hatanaka *et al.* (1974). The potassium salts of D-glucose 3-sulphate, 2-acetamido-2-deoxy-D-galactose 6-sulphate (*N*-acetyl-D-galactosamine 6-sulphate), L-fucose monosulphate (mainly 3-sulphate), 2-acetamido-2-deoxy-D-glucose 6-sulphate (*N*-acetyl-D-glucosamine 6-sulphate) and 2-sulpho-amino-2-deoxy-D-glucose (D-glucosamine *N*-sulphate) were kindly provided by Professor K. S. Dodgson and Dr. A. H. Olavesen of University College, Cardiff, U.K. Sulphoglycerogalactolipid and cerebroside 3-sulphate were prepared as described by Hatanaka *et al.* (1976*a*).

Arylsulphatase and glycosulphatase of *C. lampas*

Arylsulphatase and glycosulphatases I and II were prepared from the liver of *C. lampas* by the method of Hatanaka *et al.* (1976*b*). Specific activities of arylsulphatase towards *p*-nitrophenyl sulphate and

nitrocatechol sulphate were respectively 257 and 497 nkat/mg of protein. Glycosulphatase I (specific activity towards D-glucose 6-sulphate, 46.7 nkat/mg of protein) was generally used as glycosulphatase source in the present work, but there appears to be little or no difference in its substrate specificity from that shown by glycosulphatase II.

Determination of enzyme activities

Buffer solutions used for enzyme assays were prepared as follows. Sodium acetate/acetic acid buffer was prepared by mixing solutions of sodium acetate and acetic acid of the same concentration, so that the molarity expresses the concentration of acetate ion. Tris/HCl and Tris/acetic acid buffers were prepared by adjusting the pH of a solution of Tris base with HCl or acetic acid and then diluting to a defined molarity.

Enzyme activities towards *p*-nitrophenyl sulphate and nitrocatechol sulphate were measured by modifying the procedures of Dodgson & Spencer (1957). The reaction mixture, in a total volume of 300 μ l, contained 3 μ mol of Tris/acetate buffer, pH 7.5, and enzyme. After incubation for 1 h at 37°C, 0.5 ml of 1 M-NaOH was added, and E_{400} (*p*-nitrophenol) and E_{510} (4-nitrocatechol) were measured with the Zeiss PMQ II spectrophotometer.

Enzyme activity towards ascorbate 2-sulphate was determined by the method of Hatanaka *et al.* (1974), and that towards UDP-*N*-acetyl-D-galactosamine 4-[35 S]sulphate by the following modification of the procedure of Tsuji *et al.* (1974). The reaction mixture, in a total volume of 50 μ l, contained 10 nmol (about 1000 c.p.m.) of UDP-*N*-acetyl-D-galactosamine 4-[35 S]sulphate, 3 μ mol of sodium acetate/acetic acid buffer, pH 5.0, and enzyme. After being incubated for 15 h at 37°C the reaction mixture was placed in a boiling-water bath for 1 min. A portion (25 μ l) was then subjected to paper electrophoresis (Whatman 3MM paper) for 50 min at a potential gradient of 20 V/cm in the presence of 0.05 M-ammonium acetate/acetic acid buffer, pH 5.0. The inorganic $^{35}\text{SO}_4^{2-}$ spot was detected by the $\text{BaCl}_2/\text{rhodizonate}$ method of Schneider & Lewbart (1956), before being cut out and counted for radioactivity (Beckman LS-250 liquid-scintillation spectrometer) in the presence of 5 ml of a toluene scintillation fluid containing 4 g of 2,5-diphenyloxazole/l and 0.1 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene/l.

Enzyme activities towards other sugar sulphates were determined essentially by the method reported by Hatanaka *et al.* (1975a) for the assay of glycosulphatase. The reaction mixture (total volume 200 μ l) contained 5 μ mol of sulphate ester, 20 μ mol of sodium acetate/acetic acid buffer, pH 5.5, and enzyme. After incubation for 1 h at 37°C, 0.4 ml of 0.15 M-HCl was added, followed by 0.4 ml of the $\text{BaCl}_2/\text{gelatin}$

solution of Dodgson (1961a). The resulting turbidity was measured at 360 nm in the Zeiss PMQ II spectrophotometer. In some cases, where enzyme activity was low, enzyme reaction mixtures were incubated overnight.

Enzyme activities towards oestradiol 3-sulphate and cortisone 21-sulphate were determined by the procedure used for glycosulphatase, except that the sulphate esters were present at a concentration of 1.4 μ mol per incubation mixture. Tris/acetic acid buffer (20 μ mol) was at pH 7.0.

Enzyme activities towards sulphogalactolipids were measured as described by Hatanaka *et al.* (1975b).

Results and Discussion

Table 1 shows the activities of glycosulphatase and arylsulphatase towards a variety of carbohydrate sulphate esters. The arylsulphatase preparation exhibited appreciable activity towards ascorbate 2-sulphate and the sulphogalactolipids, none of which were significantly hydrolysed by glycosulphatase. In contrast, D-glucose 3-sulphate, D-glucose 6-sulphate and *N*-acetyl-D-glucosamine 6-sulphate were good substrates for glycosulphatase but not for arylsulphatase.

The optimum pH for glycosulphatase activity towards these three sugar sulphates was virtually identical at pH 5.5, and normal Michaelis-Menten kinetics [plotted as described by Lineweaver & Burk (1934)] were exhibited towards each of them. Kinetic constants for the enzyme acting on the three substrates are shown in Table 2. Activity towards the substrates declined in the order D-glucose 6-sulphate > *N*-acetyl-D-glucosamine 6-sulphate > D-glucose 3-sulphate, whereas affinity of enzyme for substrate increased in the order D-glucose 3-sulphate < D-glucose 6-sulphate < *N*-acetyl-D-glucosamine 6-sulphate. Values obtained by Dodgson (1961b, and personal communication) for a partially purified glycosulphatase preparation from the marine mollusc *Littorina littorea* are also recorded in Table 2 for the purpose of comparison.

D-Galactose 6-sulphate, D-galactose 4-sulphate, 6-methyl-D-galactose 4-sulphate and *N*-acetyl-D-galactosamine 6-sulphate were not hydrolysed by purified *C. lampas* glycosulphatase or arylsulphatase, although crude extracts of *C. lampas* liver were shown, in separate experiments, to hydrolyse D-galactose 6-sulphate slowly. Work by Soda (1936) suggested that such extracts exhibited considerable activity towards that substrate, and the reason for this discrepancy is not clear. The partially purified glycosulphatase preparation from *L. littorea* was also able to hydrolyse D-galactose 6-sulphate, although at only 20% of the rate at which it hydrolysed the corresponding D-glucose isomer (Dodgson, 1961b). Collectively these observations may mean that

separate enzymes are responsible for the hydrolysis of the 6-sulphates of D-glucose and D-galactose. In this connexion Takahashi & Egami (1961) showed that the chondrosulphatase of *C. lampas* is an inducible enzyme, in contrast with cellulose polysulphatase, which is constitutive. It is possible that an inducible enzyme active towards D-galactose 6-sulphate, the tissue concentration of which might therefore be very variable, might be responsible for these observed discrepancies. It is not without interest that the mould *Trichoderma viride*, when grown on either D-glucose 6-sulphate or D-galactose 6-sulphate as sole source of carbon and sulphur, produces an active enzyme system which can liberate sulphate from either sulphate ester. No enzyme

active towards D-glucose 3-sulphate is produced (Lloyd *et al.*, 1968).

UDP-N-acetyl-D-galactosamine 4-sulphate, regarded as a natural substrate for mammalian arylsulphatase B (Fluharty *et al.*, 1975), was not hydrolysed by either the arylsulphatase or the glycosulphatase of *C. lampas*. On the other hand, cerebroside 3-sulphate and sulphoglycerogalactolipid, in which the ester sulphate group is present at position 3 of a D-galactose residue and which are natural substrates for mammalian arylsulphatase A (Mehl & Jatzkewitz, 1968), are both readily hydrolysed by *C. lampas* arylsulphatase. Oestradiol 3-sulphate, a natural substrate of mammalian arylsulphatase C (now known to be an oestrogen sulphatase; see Dolly *et al.*, 1972), was also hydrolysed by *C. lampas* arylsulphatase (pH optimum, 7.0; specific activity, 46.0 nkat/mg of protein) but not significantly by the glycosulphatase. Similarly, cortisone 21-sulphate was a substrate for the former enzyme (specific activity, 4.28 nkat/mg of protein) but not by the latter. It is not clear whether activity towards this substrate merely reflects contamination of the arylsulphatase preparation with a steroid sulphatase. D-Glucosamine N-sulphate (N-S bond) was not a substrate for glycosulphatase.

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Table 1. Enzyme activities for the hydrolysis of various sugar sulphate derivatives by arylsulphatase and glycosulphatase purified from the liver of *C. lampas*

Experimental conditions are given in the text.

Sugar sulphate	Specific activities (nkat/mg of protein)	
	Aryl- sulphatase	Glyco- sulphatase
Ascorbate 2-sulphate	5.85	<0.01
Fucose sulphate	<0.1	<0.1
D-Glucose 6-sulphate	0.80	46.8
D-Glucose 3-sulphate	0.32	9.88
D-Galactose 6-sulphate	<0.1	0.1
D-Galactose 4-sulphate	<0.1	<0.1
6-Methyl-D-galactose 4-sulphate	—*	<0.1
N-Acetyl-D-glucosamine 6-sulphate	0.40	26.8
N-Acetyl-D-galactosamine 6-sulphate	<0.1	<0.1
Glucosamine N-sulphate	<0.1	<0.1
UDP-N-acetyl-D-galactosamine 4-sulphate	<0.002	<0.002
Cerebroside 3-sulphate	2.27	0.004
Sulphoglycerogalactolipid	4.97	0.003

* Not determined.

Table 2. Kinetic constants for *C. lampas* glycosulphatase acting on D-glucose 6-sulphate, D-glucose 3-sulphate and N-acetyl-D-glucosamine 6-sulphate

The values in parentheses refer to the glycosulphatase of *L. littorea* (Dodgson, 1961b, and personal communication) and have been included for the purpose of comparison.

Sugar sulphate	Optimum pH	K_m (mM)	V (nkat/mg of protein)	Relative activity
D-Glucose 6-sulphate	5.5 (5.5)	25.0 (17.0)	88.7	4.0 (10.0)
D-Glucose 3-sulphate	5.5 (5.8)	29.4 (30.0)	22.0	1.0 (1.0)
N-Acetyl-D-glucosamine 6-sulphate	5.5 (5.6)	11.8 (15.0)	39.7	1.8 (7.0)

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