Studies on Sulphatases

23. THE ENZYMIC DESULPHATION OF TYROSINE *O*-SULPHATE*

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Suzuki, Takahashi & Egami (1957) have shown that the sulphated polysaccharide (charonin sulphate) of the mucous gland of *Charonia lampas* (*Triton nodiferus*) can be sulphated by a system comprising an arylsulphate, an arylsulphatase, the carbohydrate acceptor and at least one unknown factor. The physiological significance of this type of system is not yet clear, but if similar systems occur in other organisms it would seem reasonable to expect the presence in tissues of arylsulphates whose specific role would be that of sulphate donors. There are already indications (F. Egami, personal communication) of the presence of such an arylsulphate in the mucous gland of *Charonia*.

Although arylsulphates are normally present in appreciable amounts in mammalian urine, where they appear as end products of the metabolism of phenolic materials, they have not, with one notable exception, been shown to occur as normal constituents of mammalian tissues. The exception, tyrosine *O*-sulphate, has been shown by Bettelheim (1954) to be present in one of the two peptides which are released from bovine fibrinogen by the action of thrombin. Tyrosine *O*-sulphate is always present in appreciable amounts in normal urine (Tallan, Bella, Stein & Moore, 1955). It seemed to the present authors that tyrosine *O*-sulphate might conceivably act, *in vivo*, as a sulphate donor in a manner similar to that suggested by Suzuki *et al.* (1957), and it was therefore of interest to investigate the ability of the compound to act as a substrate for various arylsulphatase enzymes. A preliminary report of this work has already been made (Dodgson, Rose & Tudball, 1957).

MATERIALS AND METHODS

Substrates

The potassium salts of *p*-acetylphenyl ulphate (APS), *p*-nitrophenyl sulphate (NPS) and phenyl sulphate (PS) were prepared by the method of Burkhardt & Lapworth (1926). Dipotassium 2-hydroxy-5-nitrophenyl sulphate (nitrocatechol sulphate, NCS) was prepared by the method of Roy (1953) as modified by Dodgson & Spencer (1956).

Tyrosine O-*sulphate*. The method described by Tallan *et al.* (1955) was modified slightly. Sulphuric acid (11 ml., sp.gr. 1·86) was stirred mechanically at −5° and L-tyrosine (2 g.) was added in small portions over a period of 20 min., great care being taken to avoid lumping. Stirring was then continued at −5° for a further period of 30 min. The reaction mixture was poured into 300 ml. of crushed ice and water containing 70 g. of $Ba(OH)_2$. Precipitated $BaSO_4$ was removed, and excess of Ba^{2+} ions were precipitated by addition of solid CO_2. After removal of $BaCO_3$ the solution was evaporated to dryness *in vacuo* at 40°, and the residue was dissolved in 60 ml. of water and passed through a column (8 cm. × 1 cm.) of Dowex 50 ion-exchange resin (Dow Chemical Co., Michigan, U.S.A.; 20–50 mesh, H^+ form). The eluate was adjusted to pH 5 with 5% KOH, concentrated to dryness *in vacuo* at 35°, dissolved in 2 ml. of water and treated alternately with small portions of ethanol and ether until 20 ml. of ethanol and 18 ml. of ether had been added. After 18 hr. at 0° the crystals of monopotassium tyrosine *O*-sulphate dihydrate were separated, and washed with 10 ml. of ethanol followed by 10 ml. of ether and dried *in vacuo*. Yield, 0·4 g. (Found: ester SO_4^{2-}, 28·1; K, 11·6. $C_9H_{10}O_8NSK,2H_2O$ requires ester SO_4^{2-}, 28·6; K, 11·6%). Ester SO_4^{2-} was determined as $BaSO_4$ after hydrolysis of the compound for 2 hr. at 100° with N-HCl. Potassium was determined by flame photometer. Loss in weight on drying at 110° *in vacuo* in the presence of P_2O_5 corresponded to $2H_2O$.

*Glycyl-*L*-tyrosine* O-*sulphate*. The method of preparation was essentially as described above except that 0·5 g. of glycyl-L-tyrosine (L. Light and Co.) was added over a period of 15 min. to 5 ml. of H_2SO_4 (sp.gr. 1·86) at −8° and stirring was continued for 25 min. The procedure described previously was then followed except that the residue was taken up in 30 ml. of water before passage through the Dowex column. Yield, 0·1 g. (Found: ester SO_4^{2-}, 23·3; K, 9·7. Monopotassium glycyl-L-tyrosine *O*-sulphate trihydrate (new compound), $C_{11}H_{13}O_7N_2SK,3H_2O$, requires ester SO_4^{2-}, 23·4; K, 9·5%). Loss in wt. at 110° corresponded to $3H_2O$.

Tyramine O-*sulphate*. Tyramine hydrochloride (L. Light and Co.) was dissolved in water and the free base precipitated by the addition of the requisite amount of K_2CO_3. The material was separated at the pump, washed with a little water and dried *in vacuo*.

The sulphation procedure was then exactly as described for glycyl-L-tyrosine *O*-sulphate except that, after passing through the Dowex column, the pH of the eluate was adjusted to 10·6 with 30% (w/v) KOH before concentrating. (Found: ester SO_4^{2-}, 32·4; K, 13·1. Monopotassium tyramine *O*-sulphate dihydrate (new compound), $C_8H_{10}O_4NSK,2H_2O$, requires ester SO_4^{2-}, 33·0; K, 13·1%). Loss in wt. at 110° corresponded to $2H_2O$.

* Part 22: Baum & Dodgson (1958).

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Paper chromatography. The homogeneity of preparations of tyrosine *O*-sulphate, glycyl-L-tyrosine *O*-sulphate and tyramine *O*-sulphate was checked by descending-paper chromatography on Whatman no. 1 paper, the solvent system being butan-1-ol–acetic acid–water in the proportions 125:30:125. After 16 hr. the chromatograms were dried and developed by spraying with a 0·25% solution of ninhydrin in acetone. R_F values of the esters and of the parent phenols are given in Table 1.

Ultraviolet-absorption spectra. It is known that sulphation with chlorosulphonic acid of compounds containing both phenolic hydroxyl and amino groups results in the sulphation of both groups. On the other hand, sulphation with H_2SO_4 at low temperature leaves the amino grouping unaffected although, under certain conditions, sulphonates may be formed. With the *O*-sulphates of tyrosine, glycyl-L-tyrosine and tyramine, it seems clear that the phenolic hydroxyl group only had been sulphated. Thus the analytical results reveal the presence of only one sulphate group per molecule and the compounds gave positive ninhydrin reactions indicating that the amino groupings were free. Desulphation was readily achieved under conditions (N-HCl at 100°) where sulphonates are stable and moreover all the compounds were readily desulphated by the arylsulphatases of Taka-diastase and *Alcaligenes metalcaligenes*.

The u.v.-absorption spectra (Fig. 1) also suggested that the compounds were true arylsulphates. It will be observed that each of the parent phenols possesses a characteristic absorption band in the region of 295 mμ, which can be attributed to the presence of the free phenolic grouping. The intensity of this band is greatly decreased in the corresponding sulphated compound indicating that sulphation of the phenolic group had occurred rather than ring sulphonation or sulphation of the amino group.

Table 1. *Chromatographic mobilities of* L-*tyrosine, glycyl-*L-*tyrosine and tyramine and their corresponding* O-*sulphate esters*

R_F values were obtained with butan-1-ol–acetic acid–water (125:30:125, by vol.). Experimental details are given in the text.

Compound	No. of experiments	Average R_F
Tyrosine	6	0·60
Tyrosine *O*-sulphate	6	0·12
Glycyl-L-tyrosine	4	0·45
Glycyl-L-tyrosine *O*-sulphate	4	0·25
Tyramine	4	0·67
Tyramine *O*-sulphate	4	0·30

Arylsulphatase preparations

A lactose-free preparation of Taka-diastase (Parke, Davis and Co. Ltd.) was used as a source of fungal arylsulphatase; the bacterial enzyme from *Alcaligenes metalcaligenes* was concentrated to give preparation B (Dodgson, Spencer & Williams, 1955) and limpet (*Patella vulgata*) arylsulphatase was concentrated to give powder D (Dodgson & Spencer, 1953*a*). Human arylsulphatases *A* and *B* were prepared from human liver according to the directions of Baum, Dodgson & Spencer (1958) and Dodgson & Wynn (1958) respectively.

Human-liver arylsulphatase C. The method of Dodgson, Spencer & Wynn (1956) was modified. Human liver (40 g.) obtained within 48 hr. after death was cut into small pieces and suspended in 150 ml. of water with the aid of a Townson and Mercer macerator. The suspension was centrifuged at 2 000 *g* (all values of *g* are average values) and 0° for 15 min. The cloudy supernatant was thrice alternately frozen and thawed before centrifuging for 30 min. at 54 450 *g* and 0° in the Spinco preparative ultracentrifuge (25 000 rev./min., head no. 30). The precipitate was washed (twice) by suspending in water and centrifuging, after which the precipitate was again suspended in water and subsequently freeze-dried.

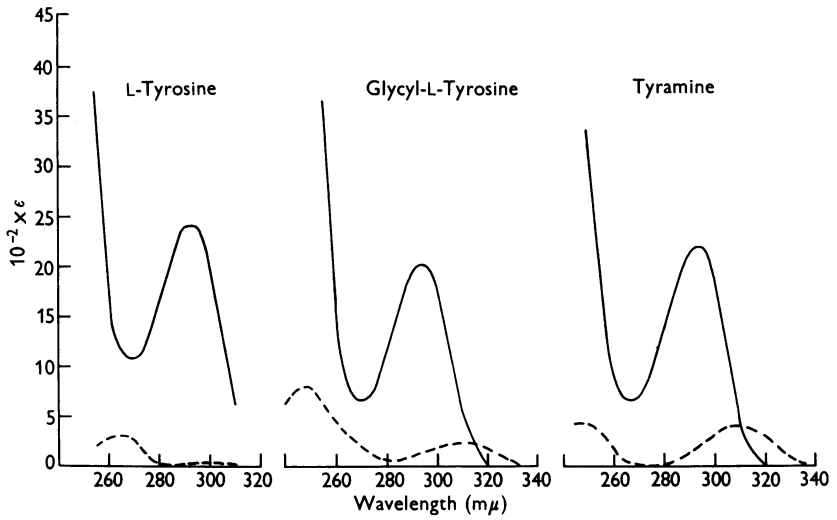

Fig. 1. Ultraviolet-absorption spectra of L-tyrosine, glycyl-L-tyrosine, tyramine and their corresponding *O*-sulphate esters. Spectra were determined in the presence of 0·1 N-NaOH with the Hilger Uvispek instrument. Continuous lines represent the spectra of the parent phenols and broken lines the spectra of the *O*-sulphates.

Rat-liver arylsulphatase C. M.R.C. hooded rats (15) were killed by a blow on the head and the livers were removed as quickly as possible. The livers were then forced through a fine wire mesh in order to remove connective tissue. Portions of the resulting pulp (total wt. 160 g.) were each suspended in 20 ml. of isotonic sucrose solution with the aid of a glass homogenizer. The final suspension (1·5 l.) was centrifuged for 10 min. at 0° and 2000 *g* and the resultant cloudy supernatant was subsequently centrifuged for 30 min. at 0° and 7700 *g* on the Spinco preparative ultracentrifuge (8750 rev./min., head no. 21). Again a cloudy supernatant was obtained and this was clarified by centrifuging for 1 hr. at 0° and 54 450 *g* (25 000 rev./min., head no. 30). The red gelatinous sediment was separated and treated with 10 g. of Lissapol-N (Imperial Chemical Industries Ltd.) before adding sufficient ice-cold water to give a final volume of 250 ml. The pH of the mixture was adjusted to 9·8 with N-NaOH and the whole allowed to stand at 0° for 30 min. The pH was readjusted to 7·5 with acetic acid and the mixture was then treated with acetone at 0° until the concentration of acetone was 80% (v/v). After standing at 0° for 30 min. to allow the precipitate to flocculate the latter was separated by centrifuging at 0°, and washed with two 150 ml. portions of cold acetone and then dried *in vacuo*. The reddish brown material (1·5 g.) was suspended in 30 ml. of ice-cold water before centrifuging on the M.S.E. refrigerated centrifuge for 1 hr. at 0° and 20 000 *g*. The sediment was washed (twice) by suspending it in 30 ml. of ice-cold water and centrifuging as before. The final precipitate was then freeze-dried.

Ox-liver arylsulphatase C. This was prepared by a modification of the method of Roy (1956). Fresh ox liver (40 g.) was cut into small pieces and suspended in 600 ml. of ice-cold water with the aid of a Townson and Mercer macerator. The suspension was centrifuged for 5 min. at 0° and 2000 *g* and the resultant cloudy supernatant was then centrifuged on the Spinco ultracentrifuge for 30 min. at 0° and 20 000 *g* (13 750 rev./min., head no. 21). The sediment was washed (twice) by suspending it in 200 ml. of ice-cold water and centrifuging as before. The sediment was finally suspended in 200 ml. of ice-cold water containing 10 g. of Lissapol-N, the pH was adjusted to 9·0 with N-NaOH and the whole allowed to stand at 0° for 1 hr. The pH of the mixture was then adjusted to 7·5 with acetic acid before centrifuging for 90 min. at 0° and 54 450 *g* (25 000 rev./min., head no. 30). The supernatant was treated with acetone at 0° until the concentration of acetone was 80% (v/v) and the precipitated material was then collected, and washed and freeze-dried as described for the rat-liver preparation.

Determination of enzyme activity

Arylsulphatase activity towards NPS and NCS was measured by spectrophotometric estimation of the respective liberated phenols (Dodgson, Spencer & Thomas, 1955). Enzyme activity towards all other substrates was determined by estimating liberated sulphate by the method of Dodgson & Spencer (1953*b*). Except where otherwise stated, incubation of enzyme (0·6 ml.) and substrate (0·6 ml.) was for 1 hr. at 37·5° in the presence of 0·5 M-sodium acetate–acetic acid buffer adjusted to the appropriate pH. With each enzyme preparation preliminary experiments were made in order to establish the concentration of enzyme appropriate to the range of accuracy of the particular assay method used.

RESULTS

Table 2 records the optimum experimental conditions for the activity of the various enzymes towards tyrosine *O*-sulphate, PS, NPS and NCS. Michaelis constants (K_m) and maximum velocities ($V_{max.} = k_3[E]$) are also given and were determined by the method (case III) of Lineweaver & Burk (1934). Values of $V_{max.}$ for any one enzyme are relative values expressed in arbitrary units since, although the concentration of enzyme used was the same in each particular series of experiments, the absolute concentration $[E]$ was unknown.

Dodgson & Spencer (1957) have previously suggested that at least two types of arylsulphatases occur in nature. The type I arylsulphatases show a relatively high activity and affinity towards simple arylsulphates such as PS and NPS and are strongly inhibited by KCN. The type II enzymes, on the other hand, whilst appreciably active towards simple arylsulphates, show relatively greater affinity and activity towards more complex arylsulphates such as NCS. They are generally unaffected by KCN but are strongly inhibited by PO_4^{3-} and SO_4^{2-} ions. Table 2 shows that the type I arylsulphatases of Taka-diastase and of *Alcaligenes metalcaligenes* both exhibit appreciable activity and affinity towards tyrosine *O*-sulphate. With *Alcaligenes metalcaligenes* the activity of the enzyme towards tyrosine *O*-sulphate is about eight times as great as that towards PS, and the Taka-diastase enzyme is about half as active towards tyrosine *O*-sulphate as towards PS. On the other hand, the type II arylsulphatase of the limpet shows only feeble affinity and activity towards tyrosine *O*-sulphate relative to that shown towards the more complex substrate NCS. The type II arylsulphatases of human liver (arylsulphatases *A* and *B*) behave similarly, although with arylsulphatase *A* it was not possible to obtain figures of absolute quantitative significance owing to the anomalous kinetics exhibited by the enzyme (see Baum & Dodgson, 1958).

The finding that the type I arylsulphatases of *Alcaligenes* and Taka-diastase were appreciably active towards tyrosine *O*-sulphate was as expected. It was therefore particularly interesting to find that the type I arylsulphatases (arylsulphatase *C*) of human, rat or ox liver were apparently devoid of activity towards this substrate although all three enzymes were readily able to attack PS.

Although the length of the incubation period and the experimental conditions of pH, substrate concentration, type of buffer and buffer concentration were widely varied, no release of SO_4^{2-} ions from tyrosine *O*-sulphate could be detected. Moreover tyrosine *O*-sulphate when tested at various concentrations (0·005–0·02 M) failed to act as a competitive

Table 2. *Experimental conditions governing the activity of various arylsulphatases towards the potassium salts of 2-hydroxy-5-nitrophenyl-, p-nitrophenyl-, phenyl- and tyrosine O-sulphate*

NCS, 2-hydroxy-5-nitrophenyl sulphate; NPS, *p*-nitrophenyl sulphate; PS, phenyl sulphate; TOS, tyrosine *O*-sulphate.

Arylsulphatase	Buffer	Concn. of buffer (M)	Optimum pH	Substrate	Optimum concn. of substrate (mM)	$10^4 K_m$	Relative $V_{max.}$	Incubation period (hr.)
Type I enzymes								
Alcaligenes metalcaligenes	Tris–acetic acid	0·2	8·0	NCS	15·0	2·2	1·2	1·0
			8·75	NPS	1·5	2·3	16·5	1·0
			8·75	PS	3·5	6·1	1·0	1·0
			9·0	TOS	10·0	3·2	7·6	1·0
Taka-diastase	Acetate	0·5	5·8	NCS	20·0	7·3	2·0	1·0
			6·3	NPS	15·0	3·7	2·0	1·0
			6·2	PS	20·0	23·0	1·0	1·0
			6·3	TOS	15·0	56·2	0·5	1·0
Rat-liver *C**	Acetate	0·5	7·4	NPS	2·5	7·0	4·0	6·0
			7·0	PS	40·0	46·0	1·0	6·0
			—	TOS	—	No activity		6·0
Ox-liver *C**	Tris–acetic acid	0·2	8·0	NPS	8·0	20·0	18·0	2·0
			8·0	PS	20·0	95·2	1·0	2·0
			—	TOS	—	No activity		16·0
Human-liver *C**	Tris–acetic acid	0·2	7·3	NPS	8·0	28·0	6·8	5·0
			7·5	PS	100·0	450·0	1·0	5·0
			—	TOS	—	No activity		16·0
Type II enzymes								
Limpet (*Patella vulgata*)	Acetate	0·5	5·5	NCS	5·0	6·9	4·5	1·0
			5·5	NPS	55·0	60·0	1·5	1·0
			5·7	PS	60·0	125·0	1·0	1·0
			5·5	TOS	60·0	180·0	0·5	1·0
Human-liver *B*	Acetate	0·5	6·0	NCS	10·0	12·3	15·0	1·0
			6·0	NPS	>50·0	124·0	1·0	3·0
			—	PS	—	No activity		16·0
			—	TOS	—	No activity		16·0
Human-liver *A*†	Acetate	0·5	5·0	NCS	5·0	—	15·7	1·0
			5·3	NPS	40·0	—	9·3	1·0
			5·3	PS	40·0	—	1·0	1·0
			5·3	TOS	40·0	—	2·6	1·0

* No figures are available for NCS. The activity shown towards NCS by these enzyme preparations can be almost completely inhibited by PO_4^{3-} ions and is therefore probably due to the presence of small amounts of arylsulphatases *A* and *B* (see Dodgson, Rose & Spencer, 1957).

† Owing to the anomalous kinetics of this enzyme it was not possible to determine Michaelis constants and the relative $V_{max.}$ figures quoted actually represent μg. of SO_4^{2-} ions liberated in 1 hr. by a fixed concentration of enzyme.

inhibitor of the hydrolysis of NPS by rat-liver arylsulphatase *C*. On the contrary, slight enzyme increases (up to 10%) in activity were observed under these circumstances. Similar results were obtained when APS was substituted for NPS, although the activatory effect of tyrosine *O*-sulphate was less pronounced then.

Experiments with soluble preparations of arylsulphatase C. Mammalian-liver arylsulphatase *C*, irrespective of the species of origin, is a microsomal enzyme which cannot be solubilized by the usual mild procedures. It therefore seemed possible that the failure of the enzyme to hydrolyse tyrosine *O*-sulphate might be related to the insoluble particulate nature of the enzyme. Accordingly, rat-liver arylsulphatase *C* was solubilized by treatment with detergents as described by Dodgson, Rose, Spencer & Thomas (1957). Three different detergents were used, the non-ionic Lissapol-N, the cationic cetylpyridinium bromide (L. Light and Co.) and the anionic Teepol 530 (Shell Chemicals Ltd.). It was not possible to test directly the activity of the solubilized enzyme against tyrosine *O*-sulphate since in each case the detergent interfered markedly with recoveries of SO_4^{2-} ions. It was therefore necessary to test the ability of tyrosine *O*-sulphate to act as a competitive inhibitor of the hydrolysis of NPS by the solubilized enzyme. These experiments were performed at pH 7·4 in the presence of a 0·0025M-solution of NPS in 0·5M-sodium acetate–acetic acid mixture (see Table 2), the concentration of tyrosine *O*-sulphate being varied between 0·005 and 0·02M. Irrespective of the length of incubation period used tyrosine *O*-sulphate did not inhibit the hydrolysis of NPS by any of the enzyme preparations. Indeed, with the Lissapol-N- and Teepol 530-treated enzyme preparations, increases of up to 10% of enzyme activity towards NPS was observed in the presence of tyrosine *O*-sulphate. It seems clear that the failure of arylsulphatase *C* to hydrolyse tyrosine *O*-sulphate is not related to the insoluble nature of the enzyme.

*Experiments with glycyl-*L*-tyrosine* O-*sulphate and tyramine* O-*sulphate*. Dodgson, Spencer & Williams (1956) have shown for the arylsulphatase of *Alcaligenes metalcaligenes* that the activity of this enzyme is markedly affected by the nature of any substituent groups present in the aromatic nucleus of the arylsulphate substrate. It was therefore of interest to see whether tyrosine *O*-sulphate could be induced to act as a substrate for arylsulphatase *C* by slightly modifying the side chain of the compound, and attempts were made to prepare various sulphate esters whose structures were closely related to that of tyrosine *O*-sulphate. The majority of these attempts were unsuccessful for a variety of reasons. Thus the ethyl and methyl esters of tyrosine could not be sulphated without loss of ethyl or methyl groups, the hydroxamic acid derivative could not be sulphated and it proved impossible to prepare the *N*-acetyl derivative of tyrosine *O*-sulphate in a form sufficiently pure for enzyme experiments. Attempts to prepare the 3-nitro- and the 3:5-di-iodo-derivatives of tyrosine *O*-sulphate were also unsuccessful. It did prove possible, however, to prepare glycyl-L-tyrosine *O*-sulphate (where the α-amino grouping of tyrosine is participating in a peptide linkage) and tyramine *O*-sulphate (which contains no carboxyl group). Glycyl-L-tyrosine *O*-sulphate was readily desulphated by *Alcaligenes metalcaligenes* or Taka-diastase at a similar rate to tyrosine *O*-sulphate. However, no hydrolysis of the compound by rat, ox or human arylsulphatase *C* could be achieved under a wide variety of experimental conditions. Tyramine *O*-sulphate was readily hydrolysed by the arylsulphatase of Taka-diastase at about twice the rate at which tyrosine *O*-sulphate was desulphated. It was also hydrolysed by rat-, ox- or human-liver arylsulphatase *C* but at an extremely slow rate. In the most favourable experiment a preparation of rat-liver arylsulphatase *C* liberated only 11·4 μg. of SO_4^{2-} ions from 0·01M-tyramine *O*-sulphate in the presence of 0·2M-2-amino-2-hydroxymethylpropane-1:3-diol (tris)–acetic acid buffer, pH 6·2, in a total incubation time of 22 hr. at 37·5°. The same concentration of enzyme liberated 457 μg. of SO_4^{2-} ions from NPS under optimum conditions in 1 hr. at 37·5°.

DISCUSSION

The occurrence of tyrosine *O*-sulphate in one of the two peptides which are released from bovine fibrinogen by the action of thrombin seems to have been established with reasonable certainty (Bettelheim, 1954), although the physiological significance of this finding is quite obscure. Tyrosine *O*-sulphate thus occupies a unique position among the naturally occurring arylsulphates, which otherwise appear to arise solely as end products of the metabolism of phenols. In another respect tyrosine *O*-sulphate appears to be a unique arylsulphate since it is reported (Segal, 1957 and personal communication) that tyrosine itself cannot be sulphated by the adenosine 3′-phosphate 5′-sulphatophosphate (3′-phosphoadenosine 5′-phosphosulphate; PAPS)–phenolsulphokinase system which is responsible for the synthesis of other arylsulphates. Workers in the Cardiff Laboratories have also attempted, without success, to sulphate tyrosine via the PAPS–phenolsulphokinase route. Now in a third respect tyrosine *O*-sulphate appears to be unique since it is completely unaffected by the mammalian arylsulphatase (arylsulphatase *C*) which might, from theoretical considerations, have been expected to hydrolyse it. Indeed, of the three known arylsul-

phatases present in mammalian liver only arylsulphatase *A* is active (feebly) towards tyrosine *O*-sulphate. It is clear that in mammals tyrosine *O*-sulphate, as such, is unlikely to be capable of acting as a sulphate donor in a system similar to that described for *Charonia lampas* by Suzuki *et al.* (1957). It is possible, of course, that sulphation of tyrosine can occur only via the PAPS–phenolsulphokinase system when both the α-amino and carboxyl groups of the tyrosine molecule are participating in peptide linkages. In a similar way it may be that blockage of both α-amino and carboxyl groups is a necessary prerequisite for the desulphation of tyrosine *O*-sulphate by mammalian arylsulphatase *C*. Attempts have been made to investigate this possibility with a sulphated specimen of β-lactoglobulin (containing about 2% of tyrosine-bound ester SO_4^{2-} ions) but the results obtained so far have been inconclusive.

Other arylsulphatases are able to desulphate tyrosine *O*-sulphate readily, the arylsulphatase of *Alcaligenes metalcaligenes* being particularly active in this respect. The activity of this enzyme towards arylsulphate esters is increased by the presence of an electrophilic substituent group (e.g. $-NO_2$) in the aromatic nucleus, as a result of the withdrawal of electrons from the environment of the ester SO_4^{2-} ion group (Dodgson, Spencer & Williams, 1956). On the other hand nucleophilic substituent groups (e.g. $-NH_2$) have the reverse effect. The fact that tyrosine *O*-sulphate is hydrolysed by the *Alcaligenes* enzyme at a rate approximately eight times as great as PS suggests that at the pH of the experiment (9·0) the strongly electrophilic carboxyl group of tyrosine *O*-sulphate, even though insulated from the aromatic ring by a two-carbon chain, is still able in some way to influence the distribution of electrons in the ester SO_4^{2-} ion grouping.

SUMMARY

1. A study has been made of the ability of tyrosine *O*-sulphate to act as a substrate for various arylsulphatase enzymes.

2. Tyrosine *O*-sulphate is not a substrate for two of the mammalian arylsulphatases (arylsulphatases *B* and *C*) and is only feebly attacked by the third mammalian enzyme, arylsulphatase *A*.

3. The failure of mammalian arylsulphatase *C* to hydrolyse tyrosine *O*-sulphate is unexpected and not in accord with theoretical predictions. The enzyme is also inactive towards glycyl-L-tyrosine *O*-sulphate and only feebly active towards tyramine *O*-sulphate.

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