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The Sulphatase of Ox Liver

5. SULPHATASE *C**

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(*Received* 7 *June* 1956)

The previous papers of this series have dealt with those sulphatases which can readily be extracted by water from an acetone-dried powder of ox liver (Roy, 1953*a*, *b*; 1954*a*) or which can be obtained in true solution by extraction of the fresh tissue with water (Roy, 1954*a*). These sulphatases, named sulphatases *A* and *B* (Roy, 1953*a*), are apparently predominantly localized in the mitochondria, at least in the liver of the mouse (Roy, 1953*a*) and of the rat (Roy, 1954*b*). They rapidly hydrolyse dipotassium 2-hydroxy-5-nitrophenyl sulphate (nitrocatechol sulphate), but attack simpler compounds such as *m*-tolyl, *p*-nitrophenyl and *p*-acetylphenyl sulphates relatively slowly. Previously unpublished work in this laboratory indicated the presence in mouse liver of another sulphatase which was localized in the microsomes, which rapidly hydrolysed *m*-tolyl and *p*-nitrophenyl sulphates, and which could not be obtained in true solution by the usual methods. Dodgson, Spencer & Thomas (1955) and Dodgson, Spencer & Wynn (1956) have shown the occurrence of comparable enzymes in rat and human tissues respectively.

The present paper describes the preparation and properties of this insoluble sulphatase, sulphatase *C*, of ox liver.

* Part 4: Roy (1955).

EXPERIMENTAL

Preparation of sulphatase C

Ox liver (200 g.) was cut into small pieces and treated in an Atomix blender (Measuring and Scientific Equipment Co. Ltd.) for 2 min. with 300 ml. of ice-cold water. All the following stages were carried out at 0° unless otherwise stated. The suspension was centrifuged for 5 min. at 2000 rev./min. and the residue of connective tissue, etc., discarded. The supernatant was then centrifuged for 30 min. at 20000 *g* and the loosely packed sediment collected. This sediment was washed twice by suspending it in water and recentrifuging.

The washed sediment was suspended in about 100 ml. of water and treated with 0·2 vol. of 10% Lissapol NX (I.C.I. Ltd.). After standing overnight the debris was centrifuged down at 35000 *g* and discarded, giving a clear solution of sulphatase *C*. The enzyme was precipitated by pouring its solution into 4 vol. of acetone at −20° and was centrifuged down at −15°: the sediment was washed successively with acetone and ether at −20° and finally dried rapidly *in vacuo* over P_2O_5. This yielded sulphatase *C* as a red-brown insoluble powder. Last traces of soluble enzymes were removed by suspending the enzyme in water, by the use of a loose-fitting glass homogenizer to obtain an even suspension, and sedimenting sulphatase *C* at 35000 *g*. The residue was washed twice with water, suspended in a convenient volume of water and freeze-dried, yielding approximately 1 g. of a buff-coloured powder which could readily be suspended in water.

The yield of sulphatase *C* activity was about 40% of that present in the original liver dispersion and represented a 40-fold concentration of the enzyme with respect to dry wt. For use in the assay described below the powder was suspended in water to give a concentration of 10 mg./ml.

Assay of sulphatase C

The method used depended upon the colorimetric determination of the *p*-nitrophenol liberated by the enzymic hydrolysis of potassium *p*-nitrophenyl sulphate, prepared by the method of Burkhardt & Lapworth (1926). To 0·2 ml. of 0·5 M 2-amino-2-hydroxymethyl-1:3-propanediol (aminotrishydroxymethylmethane)–acetic acid buffer (tris–acetate buffer), pH 8·0, was added 0·2 ml. of 0·04 M potassium *p*-nitrophenyl sulphate, followed by 0·4 ml. of the enzyme suspension. The mixture was incubated for 30 min. at 37°, with occasional shaking to keep the enzyme in suspension, and the reaction was then stopped by the addition of 3 ml. of 2% phosphotungstic acid in 0·1 N-HCl. Precipitated proteins were removed by centrifuging, and the colour was developed by pipetting 3 ml. of the clear supernatant into 5 ml. of M-Na_2CO_3. The intensity of the yellow colour was read in a Spekker absorptiometer against a suitable blank, with Ilford filter no. 601 (maximum transmission at 425 mμ.). Assays were performed in duplicate and the appropriate enzyme and substrate blanks were always included.

A few experiments were carried out with nitrocatechol sulphate as substrate. In these determinations the reaction mixture was the same as that described above except that the substrate solution was 0·08 M dipotassium nitrocatechol sulphate. The liberated 4-nitrocatechol was determined by means of the red colour developed in alkaline quinol solution (Roy, 1953*a*).

When simple aryl sulphates, such as *m*-tolyl sulphate or the naphthyl sulphates, were used as substrates the liberated phenols were determined with Folin & Ciocalteu's reagent as previously described (Roy, 1953*b*).

RESULTS

The enzymic activity was firmly bound to the cell particles and was completely insoluble in dispersions of ox liver in aqueous media. Under similar conditions at least 75% of the sulphatase *A* and *B* activity of ox liver is soluble (Roy, 1954*a*). Treatment of the first washed suspension of the enzyme with a number of detergents gave a 'solution' of sulphatase *C*. Of the detergents tested the non-ionic Lissapol NX (I.C.I. Ltd.) and Triton X-100 (Rohm & Haas Inc.) were the most effective in solubilizing the enzyme. The cationic Cetavlon (I.C.I. Ltd.) was considerably less effective and also caused an appreciable inhibition of the enzymic activity. As suggested by Spencer, Dodgson, Rose & Thomas (1955), the solubilization of the enzyme is probably due to its incorporation into the micelles of the detergent, as the enzyme is rendered completely insoluble on removal of the surface-active agent by precipitation with acetone. The sulphatase *C* of ox liver apparently resembles that of human liver (Dodgson *et al.* 1956) more than that of rat liver (Spencer *et al.* 1955) as it could not be obtained in true solution by treatment of the solubilized enzyme with crude preparations of lipase (L. Light and Co., Colnbrook, Bucks). It was also impossible to obtain a true solution of the enzyme by treatment of the insoluble preparations with *n*-butanol under a variety of conditions as described by Morton (1950).

Properties of sulphatase C

Under the conditions specified above, the hydrolysis of *p*-nitrophenyl sulphate was of zero order for times not exceeding 30 min., and with this time of incubation the reaction velocity was directly related to the concentration of enzyme. With longer times of incubation the reaction velocity decreased rapidly, apparently through denaturation of the enzyme.

The optimum substrate concentration determined at pH 8·0 was approximately 0·008–0·010 M *p*-nitrophenyl sulphate (Fig. 1) and at higher substrate concentrations the reaction velocity decreased rapidly. K_m, calculated by the method of Lineweaver & Burk (1934), was 0·002 M *p*-nitrophenyl sulphate. Both the value of K_m and the degree of substrate inhibition were very considerably influenced by changes in pH. In an attempt to obtain some information about the nature of the groups in the active centre of the molecule the variation of K_m with pH was investigated. Fig. 2 shows the results plotted according to the equations developed by Dixon (1953). The pK_m/pH plot

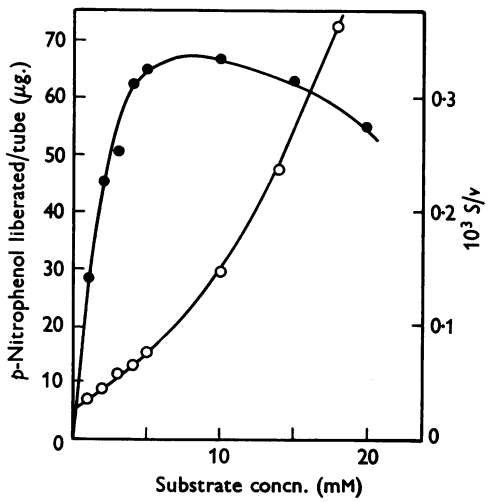

Fig. 1. Influence of substrate concentration on the reaction velocity. Volume of reaction mixture, 0·8 ml., containing 0·2 ml. of tris–acetate buffer, pH 8·0, and 0·4 ml. of sulphatase *C* suspension. Incubated for 30 min. at 37°. ●, Plot of reaction velocity (v) against substrate concentration (S); ○, plot of S/v against S.

showed a discontinuity at a pH of approximately 7·8, and according to the interpretation of these plots given by Dixon (1953) this must represent the pK of a dissociating group present either in the substrate or in the active centre of the enzyme. As *p*-nitrophenyl sulphate does not have a group which could dissociate in this region, the discontinuity must represent the pK of some group in the active centre of the enzyme. No indication of the nature of this group can be given. The fact that the slope of the pK_m/pH plot in the region above pH 7·8 is -1 indicates that in this pH range there is a change in charge of $+1$ when the enzyme combines with the substrate.

The optimum pH, determined in tris–acetate buffers at a substrate concentration of 0·01 M *p*-nitrophenyl sulphate, was 7·9–8·0 (Fig. 3). When the pH values of the buffer solutions were adjusted with HCl or H_2SO_4 no change in the position of the pH optimum was noted, and the presence of 0·08 M-NaCl or -KCl, or 0·1 M-K_2SO_4 in the reaction mixtures did not alter the position of the optimum. In phosphate buffers, on the other hand, the optimum was displaced to about pH 8·5.

With a view to distinguishing sulphatase *C* from sulphatases *A* and *B*, the action of a number of possible inhibitors was investigated. The results are shown in Table 1 and Fig. 4. In all these experiments the inhibitor was dissolved in the buffer so that the enzyme was added to the previously mixed substrate and inhibitor. The most interesting results are those summarized in Fig. 4, which shows that the inhibition of sulphatase *C* by any of a number of

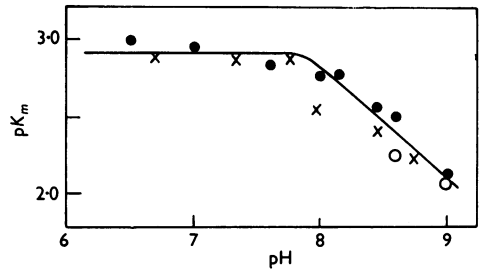

Fig. 2. Plot of pK_m against pH. Conditions as in Fig. 1. The different symbols refer to three independent experiments.

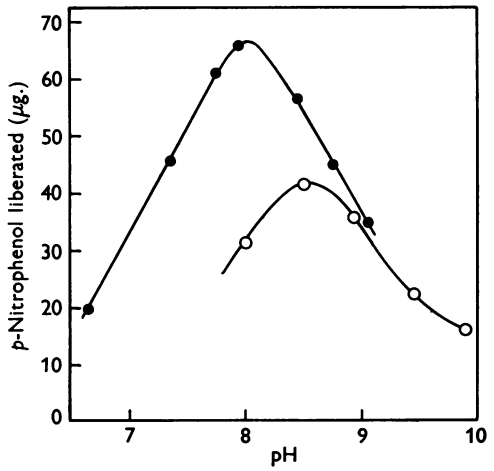

Fig. 3. Influence of pH on the reaction velocity. Volume of reaction mixture, 0·8 ml., containing 0·2 ml. of 0·04 M *p*-nitrophenyl sulphate and 0·4 ml. of sulphatase *C* suspension. Incubated for 30 min. at 37°. ●, 0·15 M tris–acetate buffers; ○, 0·01 M tris–phosphate buffers.

Table 1. *Action of possible inhibitors on sulphatase* C *activity*

The reaction mixture contained 0·2 ml. of buffer, pH 8, 0·2 ml. of 0·04 M *p*-nitrophenyl sulphate and 0·4 ml. of enzyme preparation. The inhibitors were dissolved in the buffer before use. The activity is expressed relative to control determinations with activity 1·00.

Inhibitor	Concn. (M)	Activity
Na_2SO_3	0·0001	0·45
NH_4Cl	0·05	0·80
$(NH_4)_2SO_4$	0·025	0·79
Magnesium acetate	0·05	0·64
$BaCl_2$	0·025	1·15
Barium acetate	0·025	1·10
NaF	0·01	0·55
NH_4F	0·01	0·68
KCN	0·01	0·43
Iodoacetate	0·01	0·25

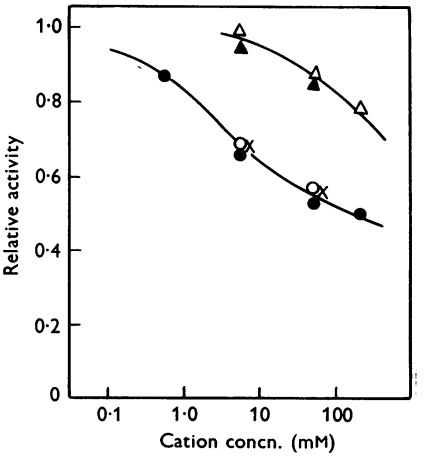

Fig. 4. Influence of various concentrations of electrolytes on the reaction velocity. Volume of reaction mixture, 0·8 ml., containing 0·2 ml. of 0·04 M *p*-nitrophenyl sulphate, 0·2 ml. of tris–acetate buffer, pH 8·0, and 0·4 ml. of sulphatase *C* suspension. ●, NaCl; ○, Na_2SO_4; ×, sodium acetate; △, KCl; ▲, K_2SO_4.

salts is due to the cation, not to the anion, Na^+ ions being considerably stronger inhibitors than K^+ ions. It should be pointed out that in the experiments with sodium salts the reaction mixtures also contained 0·01 M-K^+ ions derived from the substrate. This inhibition of sulphatase *C* by cations makes it difficult to interpret with certainty any possible action of anions, but from the results shown in Fig. 4 it seems to be clear that Cl^- and SO_4^{2-} ions can have little, if any, action on the enzyme.

The kinetics of the inhibition of sulphatase *C* by Na^+ and K^+ ions were investigated and the results are shown in Fig. 5. The inhibition by K^+ ions was non-competitive and the value of K_i was 0·7 M-K^+, as determined by the method of Lineweaver & Burk (1934). The inhibition by Na^+ ions did not correspond exactly with any of the described types of inhibition but it was clearly of the uncompetitive type. It was not, however, strictly uncompetitive as the plot of $1/v$ against $1/S$ did not give a line parallel to that of the uninhibited reaction, but parallel to that of the reaction inhibited by K^+ ions (Fig. 5). The reason for this is not clear, but it may be due to the presence of K^+ ions from the substrate in the reaction mixture. If the inhibition is assumed to be uncompetitive the value of K_i was calculated to be 0·2 M-Na^+.

The results of the study of other possible inhibitors showed little of note and are summarized in Table 1. Like all sulphatases, sulphatase *C* was strongly inhibited by SO_3^{2-} ions. It was also inhibited by cyanide but was relatively insensitive to fluoride. The inhibition by Mg^{2+} confirms the early observations of Hommerberg (1931), who was almost certainly studying the sulphatase *C* present in the unfractionated-enzyme preparations then available. The activation by Ba^{2+} ions is worthy of note. This activation cannot be due to removal of SO_4^{2-} or HPO_4^{2-} ions, which, even if present in the washed enzyme preparations used, are not inhibitors of sulphatase *C* activity. The study of inhibitors reacting with specific groups in the enzyme molecule was not undertaken, as any negative results would have had little significance in view of the very crude nature of the enzyme preparations available. Sulphatase *C* activity was, however, inhibited about 40% by 0·01 M *p*-chloromercuribenzoic acid, and this inhibition was reversed by cysteine, which was itself without action on the enzyme. These experiments were carried out under the general conditions already described (Roy, 1955) and showed that sulphatase *C*, like sulphatase *A*, is an SH enzyme.

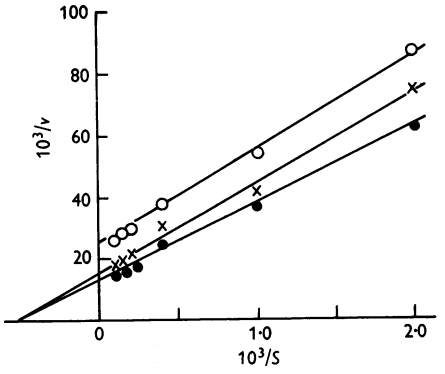

Fig. 5. Effect of NaCl and KCl on the reaction velocity. Conditions as in Fig. 4. Plotted as $1/v$ against $1/S$. ●, Control; ×, 0·1 M-KCl; ○, 0·1 M-NaCl.

Hydrolysis of other substrates

The effect of pH on rate of hydrolysis of nitrocatechol sulphate by preparations of sulphatase *C* is shown in Fig. 6. The curve shows two peaks, one at pH 5·7, the other at pH 7·5. This suggested that the preparation contained appreciable amounts of sulphatases *A* and *B*, as the pH optimum for the hydrolysis of nitrocatechol sulphate by a mixture of these enzymes is in the region of 5·7 (Roy, 1954*a*). The optimum at pH 7·5 was therefore presumably due to sulphatase *C*. This interpretation of the pH curve is supported by the results of a study of the influence of inhibitors. In the presence of fluoride, which inhibits sulphatases *A* and *B* but not *C*, the peak at pH 5·7 was suppressed, and in the presence of cyanide, which inhibits sulphatase *C* but not *A* or *B*, the peak at 7·5 was suppressed, as shown in Fig. 6.

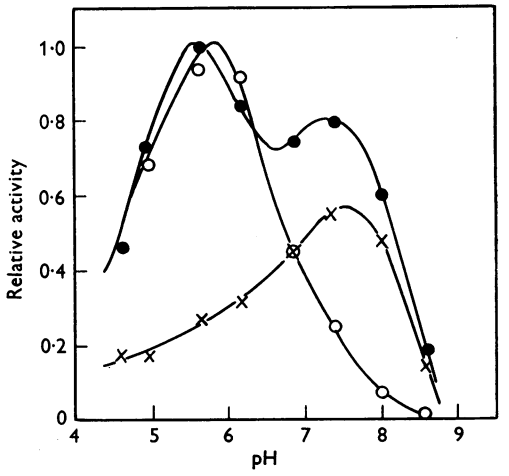

Fig. 6. Effect of pH on the hydrolysis of nitrocatechol sulphate by sulphatase *C* preparations. Conditions as in Fig. 3 except that the substrate solution was 0·08 M nitrocatechol sulphate. ●, Control; ○, 0·02 M-KCN; ×, 0·01 M-NaF.

Because of the presence of a mixture of sulphatases, all capable of hydrolysing nitrocatechol sulphate, kinetic studies on sulphatase *C* with this substrate were not carried out. The effect of substrate concentration was, however, studied at pH 5·7 and at pH 7·5. At pH 7·5 the optimum substrate concentration was 0·02M nitrocatechol sulphate and the K_m approximately 0·008M. At pH 5·7 the effect of varying substrate concentrations was comparable with the effect on a mixture of sulphatases *A* and *B* (Roy, 1954*a*).

The rate of hydrolysis of nitrocatechol sulphate at pH 7·5, at which pH sulphatases *A* and *B* are virtually inactive (Fig. 6), was approximately half that of *p*-nitrophenyl sulphate, both rates being determined at the appropriate optimum substrate concentrations. This fact readily distinguishes sulphatase *C* from sulphatases *A* or *B*, which hydrolyse nitrocatechol sulphate very much more rapidly than they do *p*-nitrophenyl sulphate (Table 3). A number of experiments suggested that the rates of hydrolysis of *m*-tolyl, 1-naphthyl and 2-naphthyl sulphates were of the same order as that of nitrocatechol sulphate, the determinations being carried out under the arbitary conditions of pH 8·0 and a substrate concentration of 0·02M.

Rat-liver sulphatase C

In view of the strong inhibition of ox-liver sulphatase *C* by Na^+ ions it seemed desirable to study the action of these ions on similar preparations of rat-liver sulphatase *C*, as Dodgson, Spencer & Thomas (1955) did not detect any inhibition of their preparations of sulphatase *C* by such cations. The enzyme was prepared from rat liver exactly as described above for ox liver.

According to Dodgson, Spencer & Thomas (1955) the optimum conditions for the assay of rat-liver sulphatase *C* are pH 8·5 with a substrate concentration of 0·006M *p*-nitrophenyl sulphate. In the present work the assays were carried out with 0·005M *p*-nitrophenyl sulphate at pH 8·0. Under these conditions no inhibition of rat-liver sulphatase *C* was caused by either Na^+ or K^+ ions, as shown in Table 2. On the contrary, in confirmation of the results of Dodgson, Spencer & Thomas (1954), a slight activation by NaCl and KCl was noted. In this respect at least, therefore, rat-liver sulphatase *C* differs slightly from the corresponding ox enzyme.

DISCUSSION

The results described above demonstrate conclusively the presence in ox liver of a third sulphatase, sulphatase *C*, strikingly different in properties from sulphatases *A* and *B*, previously described (Roy, 1953*b*, 1954*a*). These differences are summarized in Table 3. Although kinetic studies on enzyme preparations such as those described above must be interpreted with caution, as the enzyme is not in solution, it is unlikely that these differences in properties are caused by this insolubility. Apart from the fact that the properties of sulphatase *C* are not significantly altered when the enzyme is solubilized by detergents, the properties of the sulphatases *A* and *B* in these insoluble preparations are apparently no different from those of the soluble enzyme previously described. Studies on the intracellular localization of the enzyme have

Table 2. *Comparison of the influence of salts on the activity of rat-liver and ox-liver sulphatase* C

Conditions as in Table 1.

Salt	Concn. (M)	Activity: Rat	Activity: Ox
NaCl	0·05	1·20	0·56
Na_2SO_4	0·025	1·05	0·53
KCl	0·05	1·20	0·85
K_2SO_4	0·025	1·03	0·76

Table 3. *Properties of sulphatases from various sources*

The data for ox-liver sulphatases *A* and *B* are taken from Roy (1953*b*, 1954*a*), for sulphatase *C* from the present investigation, for *Aspergillus oryzae* from Robinson *et al.* (1952), and for *Alcaligenes metalcaligenes* from Dodgson, Spencer & Williams (1955). NCS, Nitrocatechol sulphate; NPS, *p*-nitrophenyl sulphate.

	Ox liver: *A*	Ox liver: *B*	Ox liver: *C*	*Aspergillus oryzae*	*Alcaligenes metalcaligenes*
K_m, NCS (mM)	0·8	70	8·0	0·35	0·22
K_m, NPS (mM)	40	25	2·0	0·17	0·47
K_i, SO_4^{2-} (mM)	0·7	70	∞	∞	∞
K_i, SO_3^{2-} (mM)	0·002	0·5	0·1	—	—
Optimum pH, NCS	4·9	6·0	7·5	5·9	8·0
Optimum pH, NPS	5·7	—	8·0	6·2	8·8
Inhibition by 0·01M-KCN (%)	2	0	60	100	69
Inhibition by 0·01M-NaF (%)	95	—	40	50*	0
Ratio of rates of hydrolysis, NCS:NPS	20	>50	0·5	0·5	0·2

* Morimoto (1938).

not been carried out because of practical difficulties in working with ox liver, and because of the fact that the above method of assay is not suited for use in the study of unfractionated-tissue preparations which reduce the liberated *p*-nitrophenol. On the basis of previous observations on the mouse, and the observations of Dodgson *et al.* (1954) on the rat, it seems that sulphatase *C* is localized entirely in the microsomes, and its extreme insolubility suggests that it must be associated with the microsomal membrane postulated by Pallade & Siekevitz (1955). It is interesting to compare this purely microsomal localization of sulphatase *C* with that of sulphatases *A* and *B*, which apparently occur in both the mitochondria and microsomes (Roy, 1954*b*, Dodgson *et al.* 1954).

The properties of sulphatase *C* are summarized in Table 3 along with those of the only other aryl sulphatases which have been studied in detail. It is clear from the data of Table 3 that sulphatase *C* is more closely related to the fungal and bacterial enzymes than to sulphatases *A* and *B*. This confirms the suggestion of Roy (1953*a*, *b*) that there are at least two groups of sulphatases in nature, and it has been suggested (Dodgson & Spencer, private communication) that the fungal and bacterial enzymes along with sulphatase *C* be called group 1 sulphatases, and the very different sulphatases *A* and *B* be called group 2 sulphatases.

The most characteristic property of sulphatase *C* is its inhibition by Na^+ ions and, to a lesser extent, by K^+ ions. This inhibition apparently distinguishes the sulphatase *C* of ox liver from that of the rat (Dodgson, Spencer & Thomas, 1953) and probably also from that of man (Dodgson *et al.* 1956). No corresponding inhibition has been noted with any soluble mammalian sulphatase (Roy, 1955) but the routine use of acetate buffers in their assay may have masked such an effect. Fromageot (1938) quotes Neuberg & Lindhardt (1923) and Neuberg & Wagner (1925) as reporting the inhibition of the sulphatase of *Aspergillus oryzae* (Taka sulphatase) by acetate buffers, although the experimental results presented in the latter two papers hardly support this statement. Dzialoszynski (1951) showed that the activity of Taka sulphatase was depressed in acetate buffers. These effects might well have been due to the cations of the acetate buffer. On the other hand, Robinson, Smith, Spencer & Williams (1952) found no inhibition of Taka sulphatase by Na^+ ions, but again this might have been due to the routine use of acetate buffers by these workers. It would therefore appear that a re-investigation of the effect of cations on the various sulphatases might be of considerable interest.

This work stresses again the danger of assaying sulphatases in unfractionated-tissue preparations. It has been stated (Dodgson, Spencer & Thomas, 1955) that the assay of sulphatase *C* in such preparations is justifiable when substrates such as *p*-acetylphenyl sulphate or *p*-nitrophenyl sulphate are used, as with these substrates competition by sulphatases *A* and *B* is negligible. The present work shows, however, that at least with ox liver, assays in such unfractionated preparations would be valueless owing to the presence of variable amounts of Na^+ ions in the preparations. Before such assays of sulphatase *C* can justifiably be carried out it is necessary to demonstrate that in the species under investigation the sulphatase *C* is uninfluenced by cations, as is apparently the case in the rat. The doubtful validity of the assay of sulphatases *A* and *B* in unfractionated-tissue preparations has already been discussed (Roy, 1954*a*; Dodgson, Spencer & Thomas, 1955).

SUMMARY

1. A method is described for the purification and assay of the sulphatase *C* of ox liver.

2. Sulphatase *C* is an extremely insoluble enzyme which has not been obtained in solution. Its properties are described. The optimum conditions for the assay of sulphatase *C* are a substrate concentration of 0·01 M *p*-nitrophenyl sulphate at pH 8·0.

3. Sulphatase *C* hydrolyses nitrocatechol sulphate at approximately half the rate of *p*-nitrophenyl sulphate.

4. Sulphatase *C* is inhibited by Na^+ ions and to a lesser extent by K^+ ions. A number of other inhibitors have been studied and it is concluded that sulphatase *C* is an SH enzyme.

5. The properties of sulphatase *C* are compared with those of other aryl sulphatases.

6. The problem of assaying sulphatases in unfractionated-tissue preparations is discussed.

The author wishes to express his gratitude to Miss Isla Sharp for her skilled technical assistance throughout this work.

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A Micro Method for the Separation and Determination of Polysaccharides by Zone Electrophoresis

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(*Received* 22 *May* 1956)

The separation and determination of the individual polysaccharides present in a mixture is a difficult and tedious process which often can only be carried out on the macro-scale. Northcote (1954) has used electrophoresis in borate buffer in the Tiselius apparatus as a method for determining the minimum number of constituents of a mixture, but the recovery of the materials behind each boundary, which is necessary if the monosaccharide constituents of each component are to be studied, is difficult. This difficulty might be overcome if the electrophoresis could be carried out on some form of strip support, and the present work describes such a technique.

Geldmacher-Mallinckrodt & Wienland (1953) have attempted to separate glycogen and galactan by electrophoresis in veronal–acetate buffer with strip supports of silk and of paper. Whilst the work to be described below was in progress, the use of filter paper as a support was investigated by Bertrand & Laszt (1956) using various buffers, and by Foster, Newton-Hearn & Stacey (1956) using borate buffer.

EXPERIMENTAL

Materials

Monosaccharides. All monosaccharides were run chromatographically and electrophoretically to establish the presence of only one reducing sugar. Concentrations of solutions were checked by polarimetry and the solutions diluted to 0·5 g./100 ml.

Polysaccharides. Those not listed below were identical with those used by Northcote (1954). Elecampane inulin, kindly given by Dr D. J. Bell, was prepared by Bell & Palmer (1952), snail galactan as by May (1934), wheat-straw xylan as by Dorée (1947) and lucerne mannogalactan as by Andrews, Hough & Jones (1952). Details of dextran A (mol.wt. 10 000) and dextran B (mol.wt. 150 000) are given by Northcote (1953). Apple pectin was a commercial sample (British Drug Houses Ltd., London).

The polysaccharides were applied as 1% aqueous solutions, except for inulin (0·5%) and ivory-nut mannan A (0·1% in 0·05M tetraborate solution). Concentrations of standard solutions of polysaccharides were checked by polarimetry. The mixed solution of polysaccharides contained 0·5 g. of each/100 ml., of yeast mannan, yeast glycogen, snail galactan and inulin, previously dried to constant weight over P_2O_5 at 100° and 0·01 mm. Hg pressure.

Methods

Apparatus. The strip support was suspended horizontally in a tank as shown in Fig. 1. The two electrode compartments each held approx. 250 ml. of buffer and the cooling fluid was tap water. The voltage applied from anode to cathode was 2000 v and since the effective length of the strip was about 40 cm. the field strength was approx. 50 v/cm. For strips 15 cm. wide, and 0·1M borate buffer, pH 9·3, typical current values were, with silk 25 mA, with paper 100 mA and with glass paper 140 mA (at 33 v/cm.). All electrophoreses were run for 90 min., except where otherwise stated. Colorimetric determinations were made in the Unicam SP. 350 colorimeter.

Buffer solutions. pH measurements were made with the glass electrode. Borate buffer was prepared from AnalaR sodium borate ('borax') and molarities were expressed in terms of sodium tetraborate ($Na_2B_4O_7,10H_2O$). Veronal

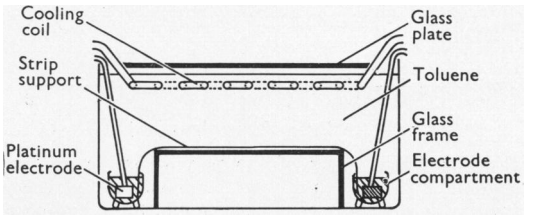

Fig. 1. Electrophoresis apparatus. Further details are given in the Experimental section.