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Purification and Properties of Aryl Sulfatases from Rabbit Sperm Acrosomes[1] (37882)

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Allison and Hartree (1) demonstrated that the detergent extract of ram sperm acrosomes contained aryl sulfatases. The localization of aryl sulfatase in the acrosome was confirmed by electron microscopy (2). The further purification and properties of spermatozoal aryl sulfatases have not been previously reported. The precise physiological function of aryl sulfatases in sperm penetration through the investments of the ovum is not yet known although it has been suggested that aryl sulfatases may be involved in the dispersal of the cumulus cells (1). We describe in this paper the purification and properties of aryl sulfatases from acrosomes of rabbit spermatozoa.

Materials and Methods. *p*-Nitrocatechol sulfate and *p*-nitrophenol sulfate were from Sigma Chemical Co., diethyl aminoethyl cellulose (DE 52) from Whatman Biochemicals Ltd., and the standard, *p*-nitrocatechol, was from Aldrich Chemical Co.

The acrosomal extract from rabbit spermatozoa was prepared by sequential treatment with $MgCl_2$ as previously described (3).

Enzyme assay. Aryl sulfatase activity was measured by following the release of *p*-nitrocatechol from *p*-nitrocatechol sulfate by a modification of the method of Roy (4). The assay mixture contained 15 m*M* substrate in 0.125 *M* acetate buffer, pH 6.0, and the enzyme in a total volume of 0.5 ml. To distinguish between aryl sulfatases A and B, 3 m*M* substrate in 0.125 *M* acetate buffer, pH 4.8, and 15 m*M* substrate in 0.5 *M* acetate buffer, pH 5.6, were used, respectively. After incubation for 1 hr at 37°, 2 ml of 2 *N* NaOH was added, and the extinction of the resulting chromophore was read at 515 nm. One unit was that amount that released one μmole of *p*-nitrocatechol in 1 min at 37°.

Acrosin was assayed using benzoyl arginine ethyl ester as the substrate (5). Hyaluronidase was assayed colorimetrically (6).

Protein was estimated by the method of Lowry *et al.* (7).

Purification. Freeze-dried acrosomal extract dissolved in 0.02 *M* Tris–HCl buffer, pH 7.4, was applied to a DEAE-cellulose column (2.5 × 34 cm) at 4° pre-equilibrated with the buffer. The column was washed with 300 ml of the buffer and eluted stepwise at 30 ml/hr with 0.1–1.5 *M* NaCl dissolved in the buffer. The aryl sulfatase fractions eluted by 0.25 *M* NaCl were pooled, dialyzed against 2 changes of 20 vol of glass-distilled water for 18 hr, and freeze-dried.

Electrophoresis. Disc gel electrophoresis was carried out at pH 7.3 on 7.5% acrylamide gel (8). Bromophenol blue was used as a tracking dye. The gels were stained in a solution of 1% amido black in 7% acetic acid and destained by using 10% acetic acid.

Results and Discussion. The purification of aryl sulfatases from rabbit sperm acrosomes is summarized in Table I. The rea-

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TABLE I. Purification of Aryl Sulfatases from Rabbit Acrosomal Extract.

Step	Total protein (mg)	Specific activity (U/mg)	Total units	Times concentrated
I. Starting material (acrosomal extract)	41.4	A 21.5	890	1
		B 16.8	695	1
II. DEAE-cellulose chromatography	6.7	A 167	1119	8
		B 202	1353	12

son for some increase in total enzyme activity with purification is not clear at this time. The increase is not due to a heat-stable inhibitor as the $MgCl_2$ extract heated at 100° for 15 min does not inhibit the purified enzyme. Similar increase in total units was observed during the purification of aryl sulfatase from chicken brain after ammonium sulfate fractionation (9). The tubes indicated by arrows (Fig. 1) show two aryl sulfatase enzyme activities, A and B, which are distinguishable by their different pH optima and substrate affinities. Aryl sulfatase C activity was not detectable in any of the fractions. Of other acrosomal enzymes, an acrosomal proteinase, acrosin, was eluted in the first peak by the buffer alone and hyaluronidase was eluted in the second peak. None of the fractions contained β-glucuronidase activity. The highly purified aryl sulfatases showed one major band on gel electrophoresis with a trace of a minor component (Fig. 2). A duplicate unstained gel was assayed for aryl sulfatase activity. The major protein band had both aryl sulfatase A and B activities.

pH optima. pH optima of the enzymes were estimated using 15 m*M* substrate by adjusting the pH with 2 *N* CH_3COOH and 2 *N* NaOH (4). The purified aryl sulfatases showed optima at pH 4.8, 5.6, and 6.0 (Fig. 3). According to Roy (10–12) aryl sulfatase A has an optimum at pH 4.9, aryl sulfatase B shows optima at pH's 5.6 and

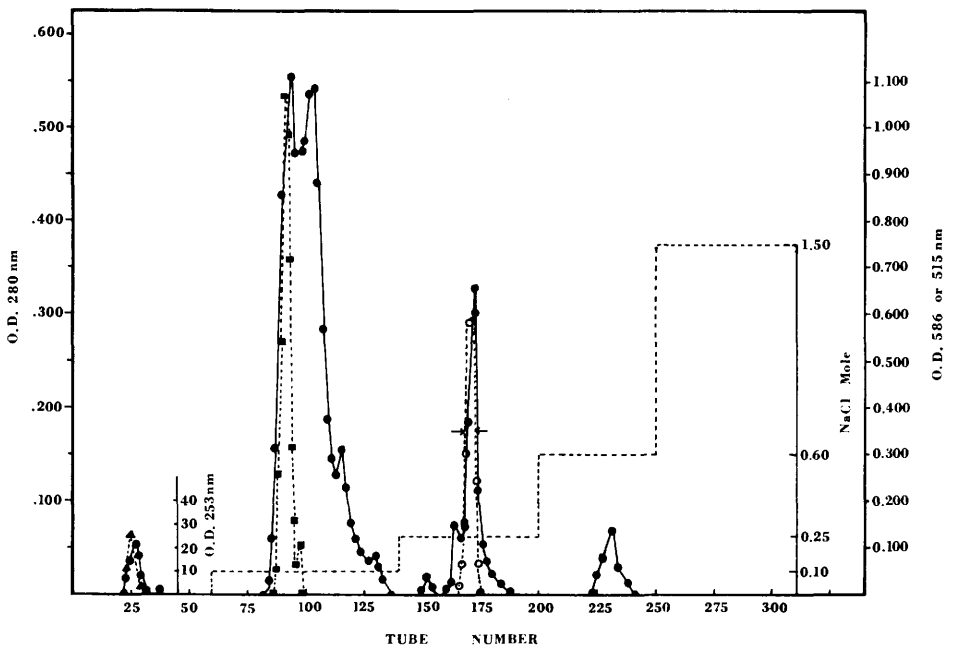

FIG. 1. DEAE-cellulose chromatography elution profile of rabbit acrosomal aryl sulfatase. The experimental details are described in the text. (-●—●-) Protein, (-▲—▲-) acrosin, (-○—○-) aryl sulfatase, (-■—■-) hyaluronidase. Aryl sulfatase activity was detected using 15 m*M* *p*-nitrocatechol sulfate in 0.125 *M* acetate buffer (pH 6.0).

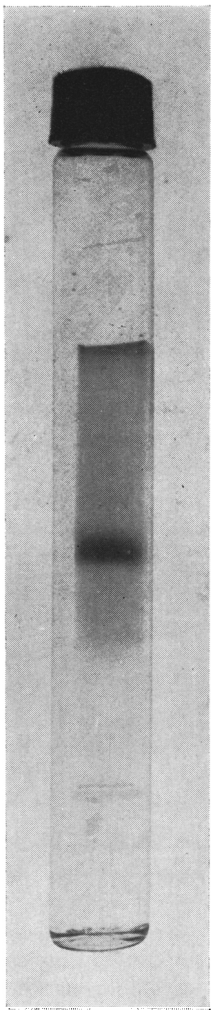

FIG. 2. Analytical gel electrophoresis of rabbit acrosomal aryl sulfatase purified by DEAE-cellulose chromatography. Sixty micrograms of protein were used per gel. Detailed procedure is described in the text.

5.9 with acetate buffer, and aryl sulfatase C has an optimum at pH 8.0 with Tris–HCl buffer in preparations from ox liver. In view of this, our purified preparation contains aryl sulfatases A and B which have nearly similar isoelectric points as judged by the disc gel electrophoresis.

Stability. Acrosomal aryl sulfatases A and B are not stable in solution at pH 7.0 stored at 4° for more than 3 days; however, they are stable for several months when stored freeze-dried. The enzymes show no activities at 4° and are inactivated on heating at 100°. The enzymes exhibit higher activities at 50° compared to 37°.

Substrate specificity and inhibition. The acrosomal aryl sulfatases are inactive towards 10 m*M* *p*-nitrophenyl sulfate both at pH's 5.0 and 6.0. Using 15 m*M* *p*-nitrocatechol sulfate at pH 6.0 as the substrate, the enzymes are completely inhibited by 25 m*M* PO_4 ions and 0.3 m*M* Na_2SO_3; the enzymes are not inhibited by 25 m*M* Na_2SO_4. KCN (10 m*M*) causes about 10% inhibition. NaCl or KCl (both 50 m*M*) does not affect the enzyme activities. According to Nicholls and Roy (13), the aryl sulfatases can be classified by the effect of sulfate ions, one of the products of the aryl sulfatase reaction. The type I aryl sulfatases are not inhibited by sulfate, whereas the type II enzymes are strongly inhibited. The non-inhibition of acrosomal aryl sulfatases by SO_4 ions is indicative of type I aryl sulfatases. On the other hand, a high affinity for *p*-nitrocatechol sulfate and little affinity for *p*-nitrophenyl sulfate indicate that the acrosomal aryl sulfatases belong to type II ac-

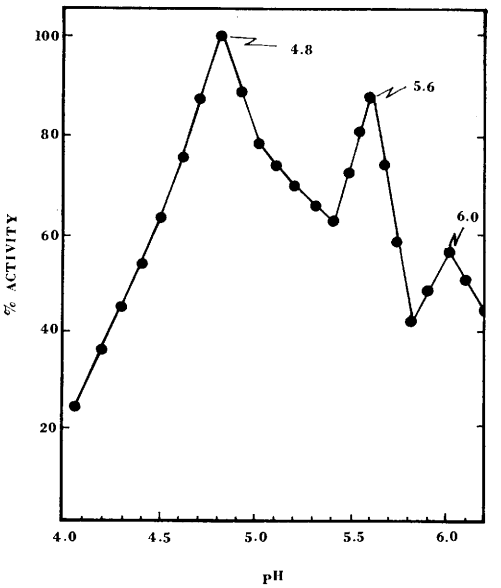

FIG. 3. pH activity curve of rabbit acrosomal aryl sulfatases. Assays were performed using 15 m*M* *p*-nitrocatechol sulfate in 0.125 *M* acetate buffer by adjusting the pH with 2 *N* CH_3COOH and 2 *N* NaOH as done by Roy (4).

cording to the classification of Dodgson and Spencer (14). Thus, acrosomal aryl sulfatases share properties of both types I and II aryl sulfatases. Aryl sulfatases are inhibited by rabbit seminal plasma pellet obtained on centrifugation at 105,000*g*. This pellet also contains decapacitation factor activity (15).

We have demonstrated high aryl sulfatase activity in acrosomal extracts of the boar, bull, rabbit, and ram spermatozoa. By sequential treatment of rabbit and ram spermatozoa, first with $MgCl_2$ followed by detergents (3), 90% of the aryl sulfatase is extractable by $MgCl_2$ along with hyaluronidase and acrosin whereas the membrane-bound enzymes, for example, sperm neuraminidase, are extracted in the subsequent detergent extract. Since $MgCl_2$ removes the outer acrosomal membrane (16), it appears that aryl sulfatases are soluble enzymes contained in the acrosomal fluid between the outer and the inner acrosomal membranes. Aryl sulfatases were released from ejaculated rabbit spermatozoa into the seminal plasma on standing at room temperature. Similar release of hyaluronidase was reported by Masaki and Hartree (17) from ejaculated bull spermatozoa.

Inadequate knowledge of the biochemical composition of investments of the ovum makes it difficult to assign a definite role for aryl sulfatases. Histochemical studies indicate the presence of sulfated mucopolysaccharides in the cumulus, the corona, and the zona pellucida of the ovum (18, 19). Further, in the mature follicle of the rabbit oocytes, there is a marked accumulation of ^{35}S between the corona cells and the zona pellucida (20, 21). Aryl sulfatases in combination with other acrosomal enzymes may facilitate sperm penetration of the ovum.

Summary. The extraction of rabbit spermatozoa with the $MgCl_2$ treatment, followed by chromatography of extracts on a DEAE-cellulose column, yield highly purified aryl sulfatases. The enzyme preparation showed one major band with the possibility of a minor contaminant by acrylamide gel electrophoresis. The single band had two activities, that of aryl sulfatases A and B. Aryl sulfatase C was not detectable. The purified aryl sulfatases had optima at pH's 4.8, 5.6, and 6.0. The enzymes exhibited no activity at 4° but exhibited higher activity at 50° than at 37°. The pure aryl sulfatases had higher affinity for *p*-nitrocatechol sulfate than for *p*-nitrophenol sulfate. Phosphate and sulfite ions inhibited the enzymes, but NaCl, KCl, and sulfate did not. Rabbit, ram, bull, and boar sperm acrosomal extracts showed high aryl sulfatase activities.

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