大韓不妊學會雜誌 : 第 10 卷 第 2 號, 1983
Kor. J. Fertil. Steril, Vol. 10, No. 2, 1983

STUDY OF ß-GLUCURONIDASE FROM SULL SEMINAL PLASMA: PURIFICATION AND PROPERTIES

Chul-Hak Yang* and Hee-Yong Lee+
**Dept. of Chemistry, College of Natural Sciences and*
+Dept. of Urology, College of Medicine, Seoul National University,
Seoul, Korea

황소의 정액에서 베타-굴룩 유로니다아제의 정제 및 그 성질에 관한 연구

서울대학교 자연과학대학화학과, 의과대학 비뇨기과학교실+
양 철 학 · 이 희 영+

(1)이 연구는 1983년도 문교부 학술연구비 보조에 의해 수행되었음.

(국문초록)

황소의 정액에서 베타 굴룩유로니다아제를 부분적으로 정제하였다. 이 정제과정에는 $(NH_4)_2SO_4$의 분획분리법, 두개의 연속적인 DEAE-셀루로오즈 콜럼 및 등전초점화법(pH4-6) 및 세파덱스 G-200의 절여과 방법이 쓰여졌다. 등전초점화(Isoelectric focusing)법을 사용했을 때 pH 5.13에서 베타 굴룩유로니다아제는 한 형태의 단백질로 존재하였다. 고도로 정제된 베타 굴룩유로니다아제는 전기영동법에 의해 한개의 주된 띠와 약간의 불순물의 띠로 나타났고 특수활동도는 34 Units/mg 단백질로 나타났다. 이 효소는 pH 5.2와 48℃에서 가장 높은 활동도를 나타냈다. 알부민이나 0.15 M 소금용액에서 베타 굴룩유로니다아제는 활동도가 상승됐다. 페놀프타레인-모노-베타-굴룩 유로닉산을 기질로 사용했을때 km 값은 2.9 mM 이었고 Vmax 값은 0.8 μ mole/min 이었다. 대두의 콘카나발린 A에 흡착하는 것으로 보아 이 효소는 당단백질임이 확인됐다. 토끼, 사람의 정자 아크롬좀 추출물 및 정액에서 이 효소는 높은 활성도를 나타냈다.

ABSTRACT

β-Glucuronidase from bull seminal plasma was partially purified by $(NH_4)_2 SO_4$ fractionation, two successive DEAE-cellulose columns, isoelectric focusing (pH 4 to 6) and Gel filtration on Sephadex G-200. Only one form of β-glucuronidase was obtained by isoelectric focusing at pH 5.13. Highly purified β-glucuronidase had specific activity of 34 units/mg protein and showed one major and some minor contaminants by disc gelk electrophoresis. The enzyme showed maximum activity at pH 5.2 and at 48°C. The enzyme was completely inhibited by 1,4 saccharo-α-lactone (5 mM). Albumin and 0.15 M NaCl increased the β-glucuronidase activity. Km of β-glucuronidase using phenolphthalein mono-β-glucuronic acid as substrate was 2.9 mM and Vmax was 0.8 μmole/min. The enzyme appeared to be a glycoprotein by its binding to concanvalin-A. Rabbit and human sperm-acrosomal extracts and seminal plasma showed high β-glucuronidase activity.

INTRODUCTION

β-Glucuronidase (β-D-glucuronide glucuronohydrolase E.C. 3.2.1.31) has been purified from various tissues, particularly from mammalian liver (Musa et al., 1965; Plapp and Cole, 1966; Delvin and Gianetto, 1970; Stahl and Tonster, 1971; Harris et al,, 1973) and preputial gland (Levvy et al., 1958; Ohtsuka and Wakabayashi, 1970). Paigen (1961) observed that a single enzyme was localized in two different subcellular structures, lysosomes and microsomes. Conchie and Mann (1957) compared the enzyme activity in the semen, seminal plasma and vesicular secretion of several mammalian species and observed that the greater amount of the enzyme was present in seminal plasma. Dott and Dingle (1968) studied the enzyme activity in the cytoplasmic droplets of bull and ram semen and observed dissociation of the enzyme from spermatozoa during maturation and ejaculation. By determining lysosomal enzyme activities, Allison and Hartree (1970) suggested that the sperm acrosome is a specialized lysosome evolved to facilitate fertilization in multicellular organisms. Recently, Zaneveld et al. (1973) and Yang and Srivastava (1975a) reported that the sperm acrosomal hyaluronidase had properties different from typical lysosomal hyaluronidase (Aronson and Davidson, 1967). The role of β-glucuronidase, a typical lysosomal enzyme, in fertilization has not been investigated as the pure enzyme was not available.

We report here the partial purification and characterization of β-glucuronidase from bull seminal plasma. As bovine liver β-glucuronidase exists in multiple forms (Plapp and Cole, 1967), the nature of seminal plasma β-glucuronidase was also examined by the isoelectric focusing technique. Rat-liver β-glucuronidase has two active components which have the same molecular weight but different electrophoretic mobilities on polyacrylamide gel electrophoresis (Delvin and Gianetto, 1970).

EXPERIMENTAL PROCEDURES

Materials. Ejaculated bull semen was received frozen from artificial breeders and was stored at -20°C until used. Whatman DEAE-cellulose (floc) was obtained from Reeve Angel Co., Inc. Sephadex G-200 in bead form was purchased from Pharmacia Fine Chemicals. Ampholine buffer (pH 4–6) was from LKB Producktor Co. Phenolphthalein-mono-β-glucuronic acid (Na-salt, lot No. 73-C-7372), phenolphthalein and sucrose were obtained from Sigma Chemical Co. The other chemicals were of reagent grade.

Methods. Enzyme assay:β-glucuronidase activity was determined by a modification of the method of Fishman et al. (1948). The reaction was carried out in a total volume of 1 ml containing 0.1 ml of phenolphthalein-mono-β-glucuronic acid (0.01 M, pH 7.0), 0.8 ml of sodium acetate buffer (0.1 M, pH 5.2), and 0.1 ml of the enzyme. After incubation for 1 hr at 37°, the released phenolphthalein showed color on addition of 5 ml of glycine-NaOH buffer (0.2 M, pH 10.4) and the chromogen was read at 540 nm. One unit equals 1 μmole of phenolphthalein released from phenolphthaleinmono-β-glucuronic acid per min at 37°. Hyaluronidase activity was measured by the modified colorimetric method (Yang and Srivastava, 1974). β-N-acetylglucosaminidase activity was determined by the method of Tarentino and Maley (1973). Protein was measured by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Preparation of seminal plasma and $(NH_4)_2SO_4$ fractionation-Bull semen was centrifuged at 3,000 x g for 10 min to remove spermatozoa. Seminal plasma was then centrifuged at 105,000 x g for 4 hr in order to remove the pellet containing the decapacitation factor (Pinsker and Williams, 1967). The supernatant from high speed centrifugation was dialyzed for 48 hr at 4° against 0.01 M sodium phosphate buffer (pH 7.1) changing the buffer at 4, 12, 24 hr. The non-dialyzable solution was brought to 30% saturation by adding solid $(NH_4)_2$ SO4 at 4° and allowing to stand overnight. The resultant supernatant was brought to 65% saturation with $(NH_4)_2SO_4$, allowed to stand overnight at 4°C, centrifuged, and the precipitate dissolved in distilled water and dialyzed against the distilled water thoroughly to remove $(NH_4)_2SO_4$. The non-dialyzable solution was lyophilized and stored at -15° in a desiccator.

Chromatography on DEAE-cellulose: Step I and Step II-Freeze-dried $(NH_4)_2SO_4$ precipitate was applied to a DEAE-cellulose column (2.5x40.5cm) at 4° equilibrated with 0.02 M sodium phosphate buffer, pH 7.3. The column was eluted with the same buffer and developed with a step-wise gradient of 0.05 M to 0.5 M NaCl solution at the rate of 23.5 ml/hr. The second and third peaks containing high β-glucuronidase activity were pooled and dialyzed against 0.02 M Na-phosphate buffer (pH 7.3) for 48 hr with three changes of the buffer. The non-dialyzable solution was concentrated by Diaflo (Amicon, Filter UM-10). Before applying the sample to the second DEAE-cellulose column, the fractions were extensively dialyzed against glass distilled water to remove salts. Dialysis against distilled water did not cause loss of enzyme activity. The concentrated enzyme solution was re-chromatographed on a second DEAE-cellulose column (2.5x27cm) by the same procedure as in the first DEAE-cellulose column.

Isoelectric focusing, Step III-Further purification was achieved by using an LKB electrofocusing column (LKB 8101, 110 ml) by the procedure outlined by the manufacturer (LKB 8100 Ampholine instruction manual). One percent Ampholine buffer (pH 4 to 6) was used with the cathode as the upper electrode. The electrode solutions were H_2SO_4 and NaOH for anode and cathode re-

spectively. The light gradient solution contained the enzyme fractions from the second DEAE-cellulose column dialyzed against 1% glycine. The power was maintained at 2-3 W for 72 hr with starting voltage of 500. Two ml fractions were collected and the pH was checked at 14°. β-Glucuronidase active fractions across the peak were pooled. The fractions were dialyzed against 0.02 M Tris-HCl buffer (pH 7.4) and concentrated with diaflo CUM-10 filter) and stored frozen.

Sephadex G-200 column chromatography, Step IV-The active fractions from isoelectric focusing column were applied to a Sephadex G-200 column (1.6x28cm) at 4° equilibrated with 0.02 M Tris-HCl buffer (pH 7.4). The column was washed with 250 ml of the buffer at the rate of 23 ml/hr. The first protein peak showing high β-glucuronidase activity was pooled and concentrated by diaflo. The concentrated enzyme fractions were stored frozen.

Acrylamide Gel Electrophoresis - Disc gel electrophoresis was carried out at pH 4.3 by the method of Brewer and Ashworth (1969) on 7.5% acrylamide gel. Bromphenol blue was used as a tracking dye. The gels were stained in a solution of 1% amido black in 7% acetic acid and were destained by 10% acetic acid.

pH optimum-For maintaining maximum buffering capacity, different buffers (0.1 M) were used through the whole pH range; HCl-KCl buffer (pH 1-2.5), Glycine-HCl buffer (pH 3-3.5), Sodium acetate buffer (pH4-5.4), Tris-Maleate (pH 5.5-7.0) and Tris-HCl buffer (pH 7.5-9.0).

RESULTS

Purification-The first centrifugation (3,000 x g) of bull semen provided seminal plasma free from spermatozoa. The decapacitation factor (Pinsker and Williams, 1967) was removed by the high speed pellet showed negligible amounts of β-glucuronidase. The 105,000 x g supernatant (80 ml) obtained from the second centrifugation contained most of β-glucuronidase activity and was used for successive purifications. Most of the contaminant proteins were removed in the supernatant at 65% saturation with $(NH_4)_2SO_4$. The data on purification are summarized in Table I.

The second peak eluted with 0.05 M NaCl and the third peak eluted with 0.1 M NaCl contained β-glucuronidase activities. Most of the β-glucuronidase appeared in the first part of the third peak as shown in Fig. 1. The first peak contained hyaluronidase and β-N-acetylglucosaminidase activities. When the DEAE-cellulose column was eluted with 0.05 M Na-phosphate buffer (pH 7.3), β-glucuronidase eluted in the first protein peak together with hyaluronidase and β-N-acetylglucosaminidase. β-Glucuronidase active fractions through the second and third protein peaks were combined and applied to a second DEAE-cellulose column to identify the multiple forms of the enzyme. As shown in Fig. 2, all of β-glucuronidase activity appeared in the second peak eluted with 0.1 M NaCl as in Step I. No hyaluronidase and β-N-acetylglucosaminidase activities were detectable across the protein peak.

Further purification and evidence for a single form was obtained by isoelectric focusing. Before applying the sample obtained from the second DEAE-cellulose column (Step II), the approximate pI value was determined using the sample obtained from Step I with Ampholine buffer pH ranging from 3.5 to 10. Isoelectric focusing column (Step III) was carried out with Ampholine buffer, pH 4 to 6 on the basis of the data obtained from the above trial. This gave better resolution compared to the broad range of pH. The pI value (pH 5.13) obtained from Step III (Fig. 3) was higher than the pI (pH 4.9) obtained in the trial with Ampholine buffer of pH 3.5 to 10. Both isoelectric focusing with Ampholine pH range 4 to 6 and pH 3.5 to 10 showed only one form of β-glucuronidase. Sucrose and Ampholine buffer (1%) used for the isoelectric focusing did not affect the enzyme activity. protein content based on O.D. 280 nm on the chromatogram of isoelectric focusing gave only approximate value since the Ampholine buffer interfered.

Gel filtration with Sephadex G-200 was used for removing sucrose and Ampholine buffer as well as the possible contaminant proteins of different sizes. Sephadex G-200 chromatography (Step IV) eliminated some of low molecular weight contaminants (Fig. 4).

Properties: Acrylamide gel electrophoresis - The final preparation of β-glucuronidase obtained by Step IV showed one major band with minor contaminant bands (Fig. 5).

pH and temperature optimum - The optimum for seminal plasma β-glucuronidase is pH 5.2 6). The enzyme has no activity at pH 1 and shows less than 10% activity above pH 7.5. The enzyme activity at 48° was 10% higher than at 37°.

Activators and inhibitors-Albumin increased the seminal plasma β-glucuronidase activity. The activity of seminal plasma β-glucuronidase increased 50% by 0.15 M and 0.3 M NaCl. The specific inhibitor of β-glucuronidase, 1,4 saccharolactone (5 mM) completely inhibited the β-glucuronidase but glucurono-γ-lactone did not. α-Chlorohydrin, a chemical male-sterilant in several mammals, strongly inhibited the seminal plasma β-glucuronidase.

Kinetics and stability-Double-reciprocal plot was used to obtain the kinetic parameters at 37° in the assay buffer. K_m and V_{max} were 2.9 mM and 0.8 μmole/min using phenolphthalein mono-β-glucuronic acid as substrate.

Crude enzyme (Step II) was stable at 4° for several months. It showed optimum activity at 48°, but lost 40% of the activity at 57° for 1 hr. The highly purified β-glucuronidase (Step IV) was not as stable when stored frozen at -15° for several weeks. Albumin stabilized the enzyme and extreme dilution affected the enzyme activity.

Molecular weight estimation-Sephadex G-200 and Sepharose 6B columns were used to determine the molecular weight of the enzyme. Seminal plasma β-glucuronidase was eluted just after horse ferritin suggesting a molecular weight of 400,000. Other standards used were ovalbumin, bovine serum albumin and pepsin.

Affinity to Con-A-Seminal plasma β-glucuronidase was tightly attached to Con-A bound to Sepharose. Since Concanavalin A is known to have specific binding sites for α-D-mannose and α-D-glucose residues (Goldstein et al., 1965), seminal plasma β-glucuronidase appears to be a glycoprotein comparable to sperm hyaluronidase (Yang and Srivastava, 1975b).

Isoelectric poing-Seminal plasma β-glucuronidase obtained by step III has only one isoelectric point at pH 5.13 with 1% Ampholine buffer pH ranging between 4 and 6.

DISCUSSION

As determined by the isoelectric focusing technique seminal plasma β-glucuronidase exists in a single form of the enzyme. By the same technique, Dean (1974) observed multiple forms of rabbit liver β-glucuronidase. Plapp and Cole (1967) reported that bovine liver β-glucuronidase exists in at least nine different forms with slight differences in carbohydrates bound to the enzyme. In our first step of DEAE-chromatography, the enzyme appeared in two different peaks. However, when the combined peaks from step I was applied to a preparative isoelectric focusing column for trial with Ampholine buffer, pH 3.5 to 10, only one protein peak containing the enzyme activity and having a single isoelectric point was observed. The localization of the β-glucuronidase in bull semen is not clear. This enzyme activity was detected in semen and seminal plasma (Conchie and Mann, 1957) and in the acrosomal preparations of bull, rabbit and human spermatozoa prepared by the method of Hartree and Srivastava (1965). Most of the enzyme activity in bull semen was found in the seminal plasma. This indicates that possibly sperm β-glucuronidase is associated with spermatozoa and released to seminal plasma after ejaculation in a manner similar to sperm hyaluronidase (Masaki and Hartree, 1962).

The pH optimum of seminal plasma β-glucuronidase is similar to that of bovine heart enzyme (Romeo and DeBernard, 1968) but is different from bovine liver enzyme (Plapp and Cole, 1966). Although Cashman et al. (1969) reported that 0.15 M NaCl inhibits β-glucuronidase from rat skin extracts. co npletely, seminal plasma β-glucuronidase was activated by 0.15 M and 0.3 M NaCl. Kushinsky et al. (1967) and Wang and Touster (1972) also observed that the enzyme was activated by NaCl. The molecular weight of bull seminal plasma β-glucuronidase is nearly the same as that of rabbit liver β-glucuronidase by gel filtration (Dean, 1974). However, the molecular weight of rabbit liver β-glucuronidase by sucrose gradient sedimentation showed remarkable discrepancies (Dean, 1974). The molecular weight of bovine liver β-glucuronidase has been estimated to be in the range of 160,000-290,000 (Buddecke and Hoefele, 1966; Plapp and Cole, 1966; Romeo and DeBernard, 1968). The possibility of discrepancies on gel filtration by virtue of the carbohydrate moiety was discussed by Andrews (1965). Affinity of seminal plasma β-glucuronidase to Concanavalin-A Sepharose indicates that the enzyme is perhaps a glycoprotein. Dean (1974) observed multiple values of pI 4.5 to 5.8 in the purified rabbit liver β-glucuronidase by isoelectric focusing. Bull sperm β-glucuronidase shows one value of pI, 5.13 with Ampholine buffer in the range of pH 4-6, i.e., the same buffer as used by Dean (1974). Ohtsuka and Wakabayashi (1970) reported that β-glucuronidase from preputial glands of the female rats has an isoelectric point 6.2. Although this enzyme is rich in bull semen, no report has been available for its purification and characterization. This report provides the method for obtaining highly purified enzyme without contamination from other sperm enzymes. α-Chlorohydrin (3-Cl,-1,2 propanediol), a male antifertility agent in the rat and several other species inhibits the seminal plasma β-glucuronidase (Yang et al)., Investigations on the mechanism of inhibition and role of the enzyme in fertilization are currently under study.

REFERENCES

Allison, A. C., and Hartree, E. F. (1970), *J. Reprod. Fert. 21, 501.*

Andrews, P. (1965). *Biochem. J. 96, 595.*

Aronson, N. N., Jr., and Davidson, E. A. (1967), *J. Biol. Chem. 242, 4.*

Brewer, J. M., and Ashworth, R. B. (1969), *J. Chem. Educ. 46, 41.*

Buddecke, E., and Hoeffele, O. (1966), *Hoppe-Seylers, Z. Physiol. Chem. 347, 173.*

Cashman, D. C., Laryea, J. U., and Weissmann, B. (1969), *Arch. Biochem. Biophys. 135, 389.*

Conchie, J., and Mann, T. (1957), *Nature, Lond. 179, 1190.*

Dean, R. T. (1974), *Biochem. J. 138, 395.*

Delvin, E., and Gianetto, R. (1970), *Biochim. Biophys. Acta 220, 93.*

Dott, H. M., and Dingle, J. T. (1968), *Exp, Cell Res. 52, 523.*

Fishman, W. H., Springer, B., Brunetti, R. (1948) *J. Biol. Chem. 173, 449.*

Goldstein, J. J., Hollerman, C. E., and Smith, E. E. E. (1965), *Biochemistry 4, 896.*

Harris, R. G., Rowe, J. J. M., Stewart, P. S., and Williams, D. C. (1973), *FEBS Letters 29, 189.*

Hartree, E. F., and Srivastava, P. N. (1965), *J. Reprod. Fert. 9, 47.*

Kushinsky, S., Chem, V. L. P., and Otterness, I. (1967), *Nature 214, 142.*

Levvy, G. A., McAllen, A., and Mash, C. A. (1958), *Biochem. J. 69, 22.*

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193, 265.*

Masaki, J., and Hartree, E. F. (1962), *Biochem. J. 84, 347.*

Musa, B. U., Doe, R. P., and Seal, U. S. (1968) J. Biol. Chem. 240, 2811.

Ohtsuka, K. and Wakabayashi, M. (1970), Enzymologia 39, 109.

Paigen, K. (1961), *Exp. Cell. Res. 25, 286.*

Pinsker, M. C., and Williams, W. L. (1967), *Arch. Biochem. Biophys. 122, 111.*

Plapp, B. V., and Cole, R. D. (1966), *Arch. Biochem. Biophys. 116, 193.*

Plapp, B. V., and Cole, R. D. (1967), *Biochemistry 6, 3676.*

Romeo, D. and DeBernard, B. (1968), *Biochim. Biophys. Acta 159, 194.*

Stahl, P. H., and Touster, O. (1971), *J. Biol. Chem. 246, 5398.*

Tarentino, A. L., and Maley, F. (1973), *Methods in Enzymol. 28, 772.*

Wang, C-C., and Touster, O. (1972), *J. Biol. Chem. 247, 2644.*

Yang, C. H., and Srivastava, P. N. (1974), *J. Reprod. Fert. 37, 17.*

Yang, C. H., and Srivastava, P. N. (1975a), *J. Biol. Chem. 250, 79.*

Yang, C. H., and Srivastava, P. N. (1975b), *Biochim. Biophys. Acta (in press).*

Yang, C. H., Srivastava, P. N., and Williams, W. L. (1975) *Fedn. Proc. Fedn. Am. Socs. Exp. Biol. 34, 255.*

Zaneveld, L. J. D., Polakoski, K. L., and Schumacher, G. F. B. (1973), *J. Biol. Chem. 248, 564.*

FIGURE LEGENDS

FIGURE 1. Elution profile of active fractions on the first DEAE-cellulose column (Step I). The column (2.5 x 40.5cm) was developed with 0.02 M Na-phosphate buffer (pH 7.3) and a stepwise gradient from 0.05 M to 0.5 M NaCl.

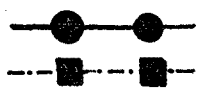


FIGURE 2. Elution profile of active fractions from the first DEAE-cellulose column rechromatographed on a second DEAE-cellulose column chromatography (Step II). The column (2.5 x 27cm) was developed with 0.02 M Na-phosphate buffer (pH 7.3) and with a stepwise gradient from 0.1 M to 0.3 M.

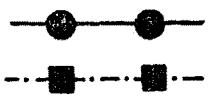


FIGURE 3. Elution diagram of active fractions from second DEAE column (Step II) subjected to isoelectric focusing column (Step III). The isoelectric focusing (column LKB 8101) was carried out in 1% Ampholine buffer (pH 4-6) on continuous sucrose gradient. The power was maintained 2-3 W for 72 hr. Detailed procedure for collection and pH measurement is described in the text.

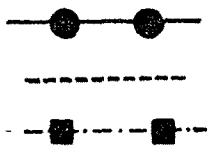


FIGURE 4. Elution profile of the active fractions from isoelectric focusing column subjected to Sephadex G-200 (1.0 x 28cm) (Step IV). The column was developed with 0.02 M Tris-HCl buffer (pH 7.4).

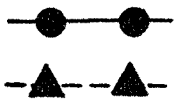
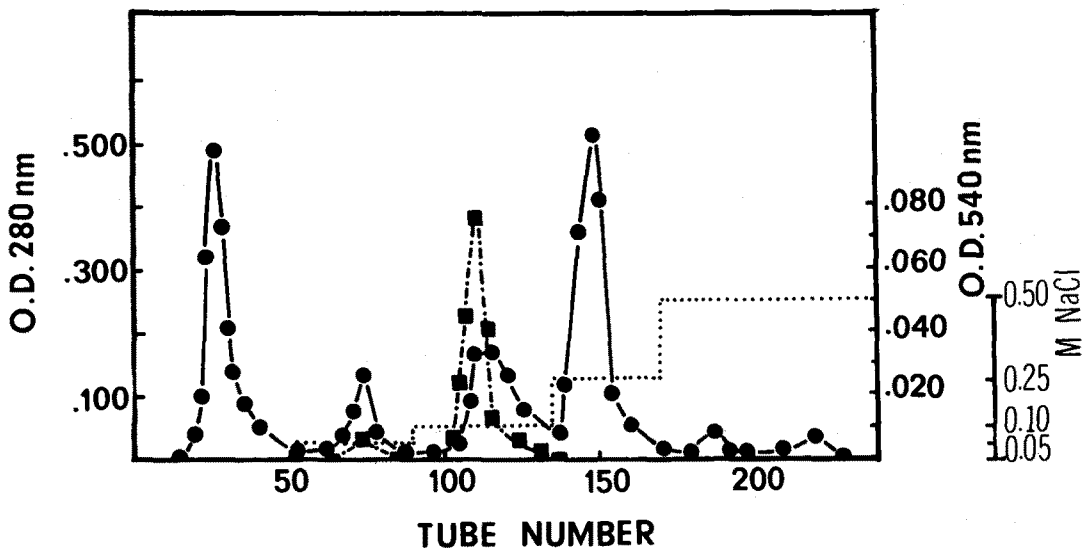
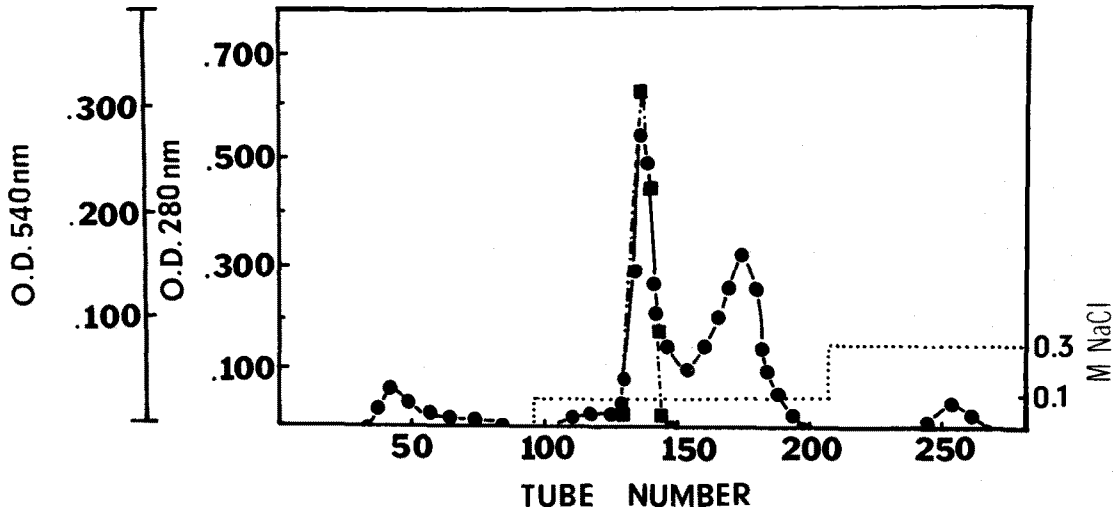
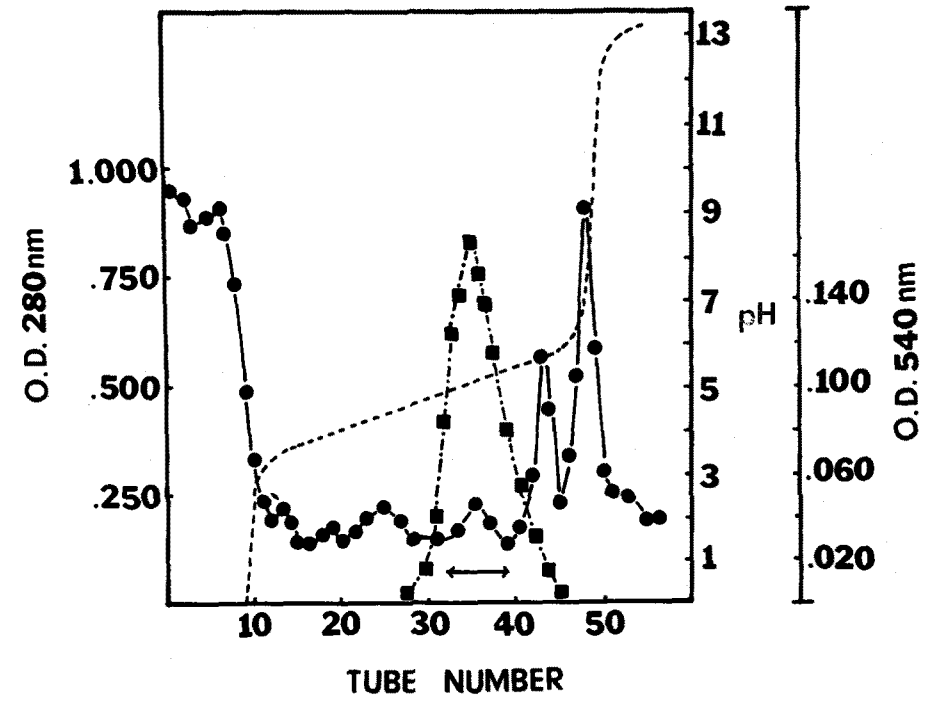


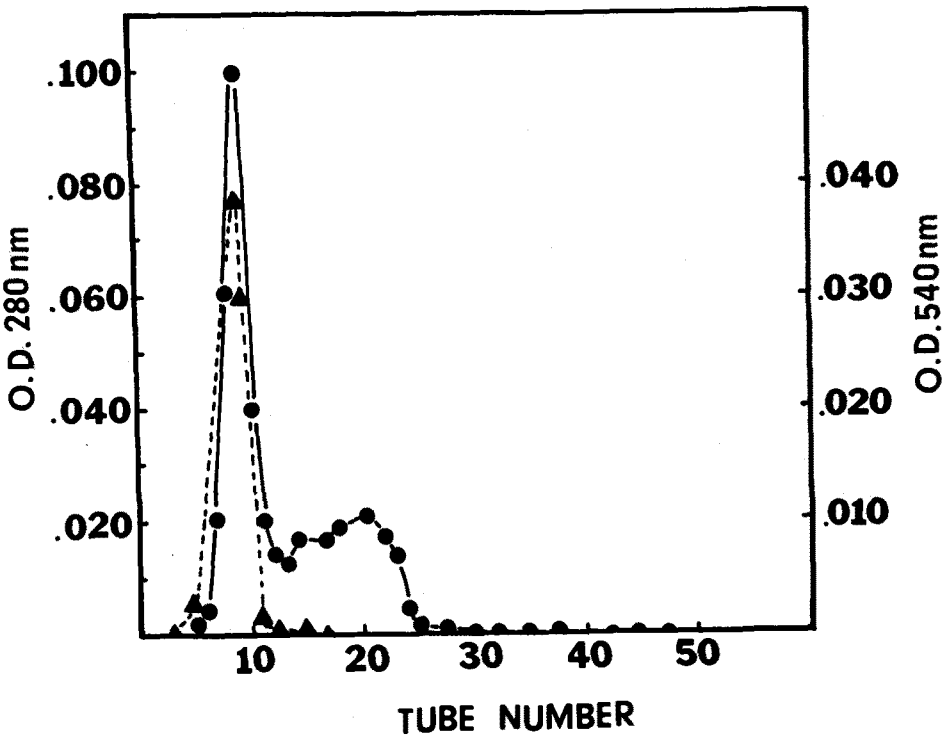
FIGURE 5. Acrylamide gel electrophoresis of final purified β-glucuronidase by Step IV. Sixty μg of protein was applied on 7.5% acrylamide gel with running pH of 4.3.

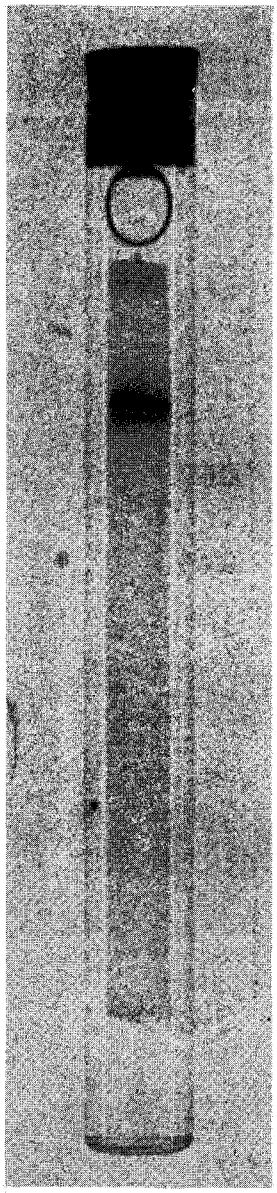
FIGURE 6. pH activity curve of bull seminal plasma β-glucuronidase. The buffers (0.1 M) were; HCl-KCl buffer (pH 1-2.5), Glycine-HCl buffer (pH 3-3.5), Sodium acetate bmffer (pH 4.0-5.4), Tris-maleate buffer (pH 5.5-7.6) and Tris-HCl buffer (pH 7.5-9.0).


O.D. 280 nm
.500
.300
.100
.080
.060
.040
.020
O.D. 540 nm
0.50
0.25
0.10
0.05
M NaCl
50
100
150
200
TUBE NUMBER


O.D. 540nm
.300
.200
.100
O.D. 280nm
.700
.500
.300
.100
0.3
0.1
M NaCl
50
100
150
200
250
TUBE NUMBER


O.D. 280nm
1.000
.750
.500
.250
13
11
9
7
5
3
1
pH
.140
.100
.060
.020
O.D. 540 nm
10
20
30
40
50
TUBE NUMBER


O.D. 280nm
.100
.080
.060
.040
.020
.040
.030
.020
.010
O.D.540nm
10
20
30
40
50
TUBE NUMBER



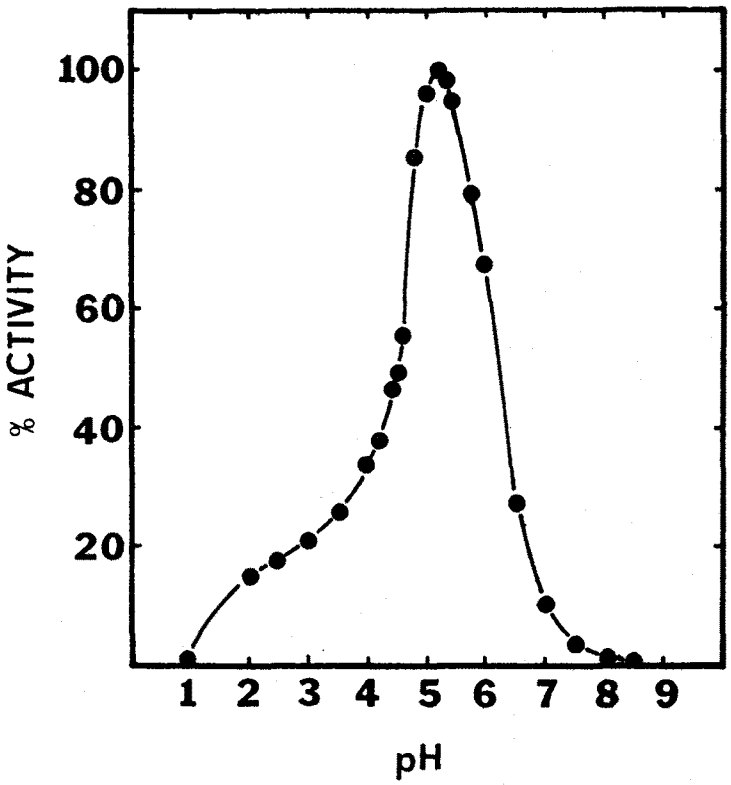

100
80
60
40
20
% ACTIVITY
1 2 3 4 5 6 7 8 9
pH

TABLE I

SUMMARY OF PURIFICATION OF β-GLUCURONIDASE FROM BULL SEMINAL PLASMA

STEP	TOTAL PROTEIN (mg)	TOTAL ACTIVITY	SPECIFIC ACTIVITY	RELATIVE ACTIVITY	YIELD %
105,000 g Super	6,215	3,729	0.6	1	100
(Bull seminal plasma)	(80 ml)				
$(NH_4)_2SO_4$ fractionation (30 – 65%)	2,035	2,095	1	1.7	56.2
I. DEAE-cellulose column first	314	785	2.5	4.2	21
II. DEAE-cellulose column second	22	96	4.3	7.2	2.5
III. Isoelectric focusing (pH 4–6)	3	43	15.5	25.8	1.1
IV. Sephadex G-200 column	0.4	14	34	57	0.4