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Note

Simple detection of steroid sulphatase activity after chromatographic fractionation

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Determination of steroid sulphatase activity has been performed by isotopic, colorimetric and enzymatic methods[1–4]. Determination of sulphatase activity by colorimetric methods, such as the methylene blue method and the Zimmermann reaction, is time-consuming because removal of the organic solvent is required, making it difficult to treat the large number of samples which are collected in chromatographic fractionation. In this paper, a one-tube detection method for steroid sulphatase activity after chromatography is described.

MATERIALS AND METHODS

All chemicals were of analytical-reagent grade and were commercially available. The different steroid substrates were from Sigma (St. Louis, MO, U.S.A.) and Ikapharm (Ramat Gan, Israel). Sephadex and the column were purchased from Pharmacia (Uppsala, Sweden). A 0.05 *M* acetate buffer (pH 5.0) was employed. The substrate solution contained 0.5 mg/ml of dehydroepiandrosterone sulphate and estrone sulphate in water. The steroid sulphatase used in these studies was sulphatase from *Helix pomatia* (Type H-1, E.C. 3.1.6.1) (Sigma) and 200 U/ml of enzyme solution was prepared. Preparation of an enzyme solution for the colour development of dehydroepiandrosterone has been described previously[4–7]: 20 mg of 4-aminoantipyrine, 100 mg of phenol, 100 U of 3β-hydroxysteroid oxidase (E.C. 1.1.3.6 from *Brevibacterium sterolicum*), 1000 U of peroxidase and 0.1 ml of Triton-X 100 were dissolved in 50 ml of phosphate buffer (0.2 *M*, pH 7.5).

Sephadex-Gel filtration

Sephadex G-200 was swollen by heating a suspension of the particles in acetate buffer (0.05 *M*, pH 5.0) for 4 h at 90°C under constant stirring. The "fines" were removed by several decantations and the slurry was poured directly into the column (60 × 1 cm) which was then washed for 3 h with acetate buffer.

Application of sample to the column

A solution of the enzyme in acetate buffer, *ca.* 200 units/ml, was centrifuged for 5 min at 2500 *g* and the supernatant of 1 ml was applied to the column. Twenty-five fractions (each 1.5 ml) were collected.

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Enzyme activity assay

Steroid sulphatase activity was detected by the use of dehydroepiandrosterone (3β-hydroxy-5-antrosten-17-one) sulphate and estrone sulphate as substrate, and β-glucuronidase activity was detected using phenolphthalein glucuronic acid as substrate.

Steroid sulphatase activity assay by the use of dehydroepiandrosterone sulphate as substrate[4]. A 0.2-ml portion of each fraction was transferred by pipette into a test tube and 0.5 ml of substrate solution and 0.8 ml of acetate buffer (0.05 *M*, pH 5.0) were added. After incubation (*ca.* 5–10 h at 37°C), 1 ml of colour development reagent for dehydroepiandrosterone was added and the mixture was incubated for 15 min at 37°C. The absorbance was measured at 500 nm.

Steroid sulphatase activity assay by use of estrone sulphate as substrate. A 0.2-ml portion of each fraction was pipetted into a test tube, and 0.5 ml of substrate solution and 1 ml of 0.2 *M* acetate buffer (pH 5.0) were added. After incubation (for 30–60 min) at 37°C, the turbidity caused by insoluble released estrone was measured at 400 nm.

β-Glucuronidase activity assay by use of phenolphthalein glucuronic acid. To 0.05 ml of sample, 0.3 ml of phenolphthalein glucuronic acid (0.003 *M*, pH 4.5) was added. After incubation for 30 min at 37°C, 1.5 ml of glycine buffer (0.2 *M*, pH 10.4) was added. The absorbance was measured at 540 nm.

RESULTS AND DISCUSSION

Gel chromatography of sulphatase from *Helix pomatia* (Type H-1) on Sephadex G-200 is shown in Fig. 1. Protein was detected at 280 nm and the steroid

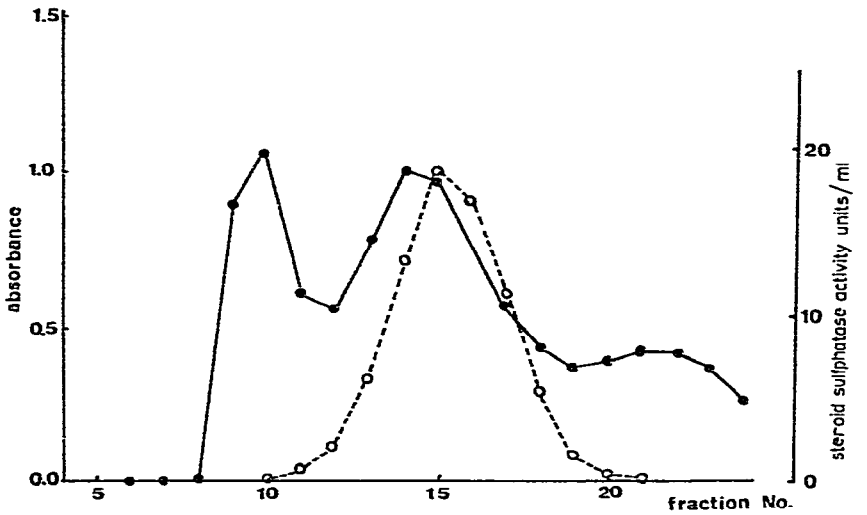

Fig. 1. Gel filtration of steroid sulphatase on Sephadex G-200. A 1-ml portion of steroid sulphatase solution (200 units/ml) from *Helix pomatia* (Type H-1) was applied onto Sephadex G-200 with 0.05 *M* acetate buffer (pH 5.0) as eluent. Fractions (each 40 ml) were collected as described in the text. Protein content was detected at 280 nm (●—●), and steroid sulphatase activity was detected by the present method (O----O; absorbance at 500 nm and calculated activity).

sulphatase activity was measured by use of dehydroepiandrosterone sulphate (absorbance at 500 nm, steroid sulphatase activity calculated) under the optimum conditions previously reported. The activity of β-glucuronidase, a contaminant of sulphatase, is shown in Fig. 2 in which the absorbance at 540 nm is plotted. Steroid sulphatase activity, measured using estrone sulphate as substrate, is also shown in Fig. 2 and was determined by turbidimetric assay (absorbance at 400 nm).

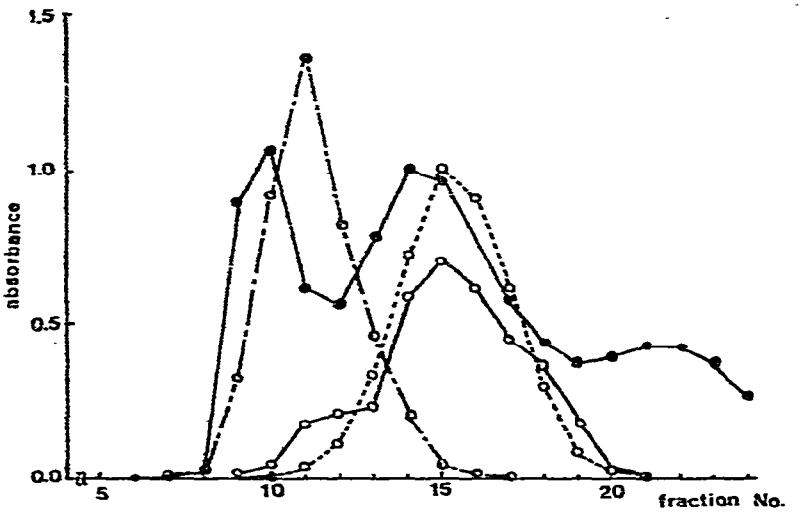

Fig. 2. Gel filtration of β-glucuronidase and steroid sulfatase on Sephadex G-200. Protein content: ●—● (absorbance at 280 nm). β-Glucuronidase: ◑- - -◑ (absorbance at 540 nm), detected by the method described in the test. Steroid sulphatase activity: O----O (absorbance at 500 nm). Steroid sulphatase activity with estrone sulphate as substrate (O—O) was measured by the method described in the text (absorbance at 400 nm).

The enzymatic determination of sulphatase activity has been reported as a preliminary communication[4]. In this paper, a simple method for determining steroid sulphatase activity after chromatography is described.

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