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Chromatographic and electrophoretic behaviour of chromogranine obtained from bovine and equine adrenal medulla

It has been shown that adrenaline and noradrenaline are contained in storage vesicles in the adrenal-medullary cells and that they can be isolated by differential centrifugation of medulla homogenates in 0.3 *M* sucrose. Osmotic lysis of the vesicles results in about 80 % of the total protein being recovered in the supernatant fraction[1–3]. A major component of these proteins has been isolated and characterized, and has been referred to as chromogranine[3–6]. This is an acidic protein, probably made up of two subunits having a molecular weight of 40,000; in the presence of ATP the chromogranine binds catecholamines although not to an extent sufficient to explain their concentration within the storage granules[4]. The present paper is a

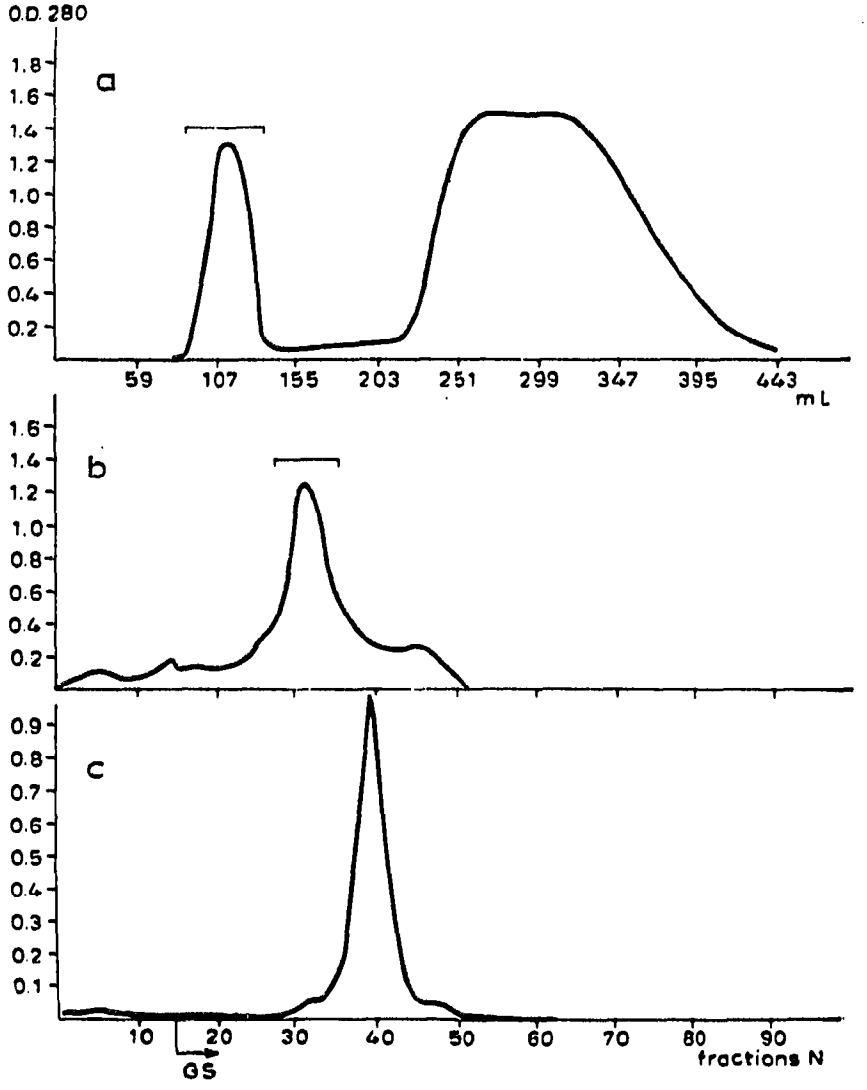

Fig. 1. Purification of chromogranine obtained from bovine adrenal medulla on (a) a 2 × 120 cm Sephadex G-25 column, (b) a 1 × 18 cm DE-52 column, and (c) a 1 × 12 cm DE-52 column. The catecholamine storage vesicles were isolated by centrifugation and then lysed by dialysis against distilled water.

report on the chromatographic and electrophoretic behaviour of chromogranine obtained from both equine and bovine adrenal medulla.

Materials and methods

Horse and calf adrenal glands were collected, fresh from the slaughterhouse. The glands were freed from connective and adipose tissue and homogenized in 0.3 *M* sucrose. After centrifugation at 1000 r.p.m. for 15 min to remove nuclei and unbroken cells, the supernatant was centrifuged at 25,000 r.p.m. for 20 min.

The granules were collected, washed twice with 0.3 *M* sucrose and finally lysed by dialysis against distilled water. After dialysis the insoluble material was removed by centrifugation at 25,000 r.p.m. The clear supernatant was then lyophilized and used for chromatography. Gel filtration was performed both on Sepharose and Sephadex columns, equilibrated with 0.05 *M* Tris–HCl buffer, pH 7.4. Ion-exchange chromatography was performed on DE-52 cellulose columns at the same pH.

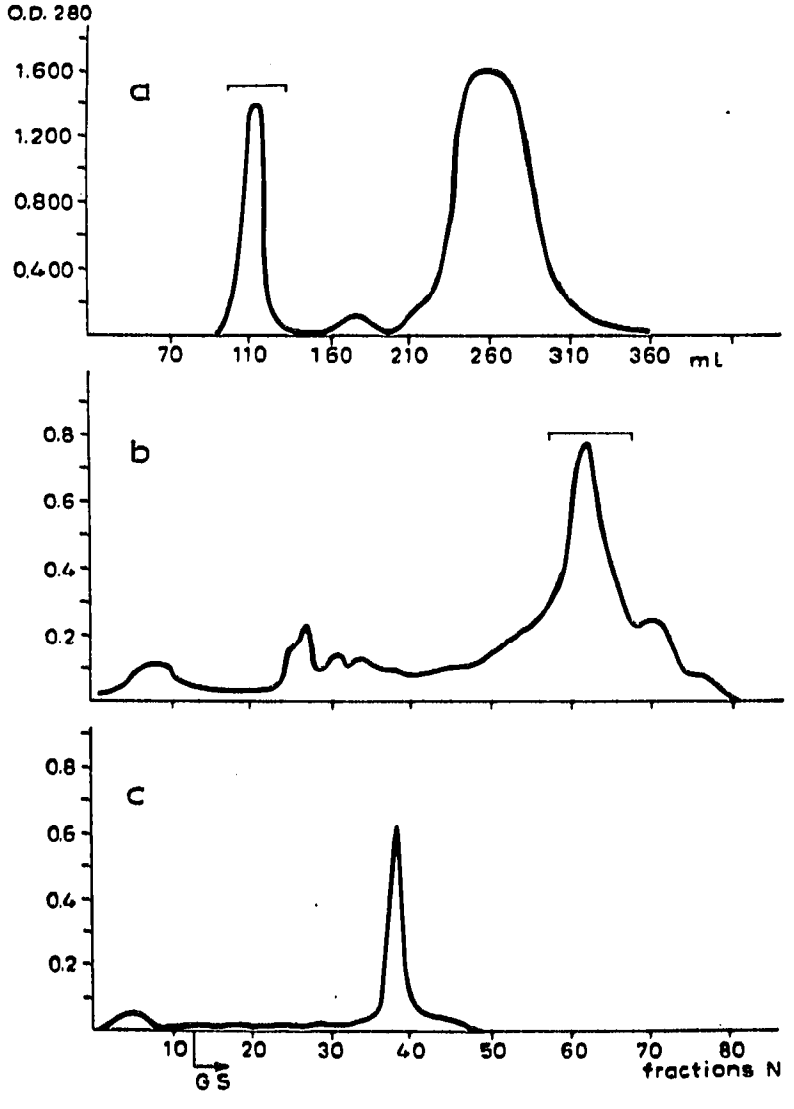

Fig. 2. Purification of chromogranine obtained from equine adrenal medulla on (a) a 2 × 120 cm Sephadex G-25 column, (b) a 1 × 18 cm DE-52 column, and (c) a 1 × 12 cm DE-52 column.

Acrylamide gel electrophoresis was carried out at pH 9.6 according to DAVIS[7]. Specific antisera to the purified chromogranines were prepared by injecting 2 mg of protein in Freund's adjuvant into rabbits. The antisera were tested by an immunodiffusion method in agar plates.

Results

Figs. 1 and 2 show the purification of chromogranine from lysates of the storage vesicles of equine and bovine adrenals. As can be seen, the lyophilized supernatant was first dissolved in water and passed through a 2 × 120 cm Sephadex G-25 column in order to separate the protein components (first peak) from the catecholamines (second peak). The protein peak was then chromatographed on a 1 × 18 cm DE-52 cellulose column equilibrated with 0.01 *M* Tris–HCl buffer, pH 7.4. A linear gradient from 0 to 1 *M* NaCl was used to elute the column. A major protein peak (chromogranine) and a few minor ones were obtained; the central part of the major peak was collected, dialyzed and re-chromatographed on a second 1 × 12 cm DE-52 column under the same conditions, and a single, symmetrical peak was obtained.

This protein peak, from both equine and bovine adrenals, was used to immunize rabbits; the specific antisera were tested in agar plates with various concentration

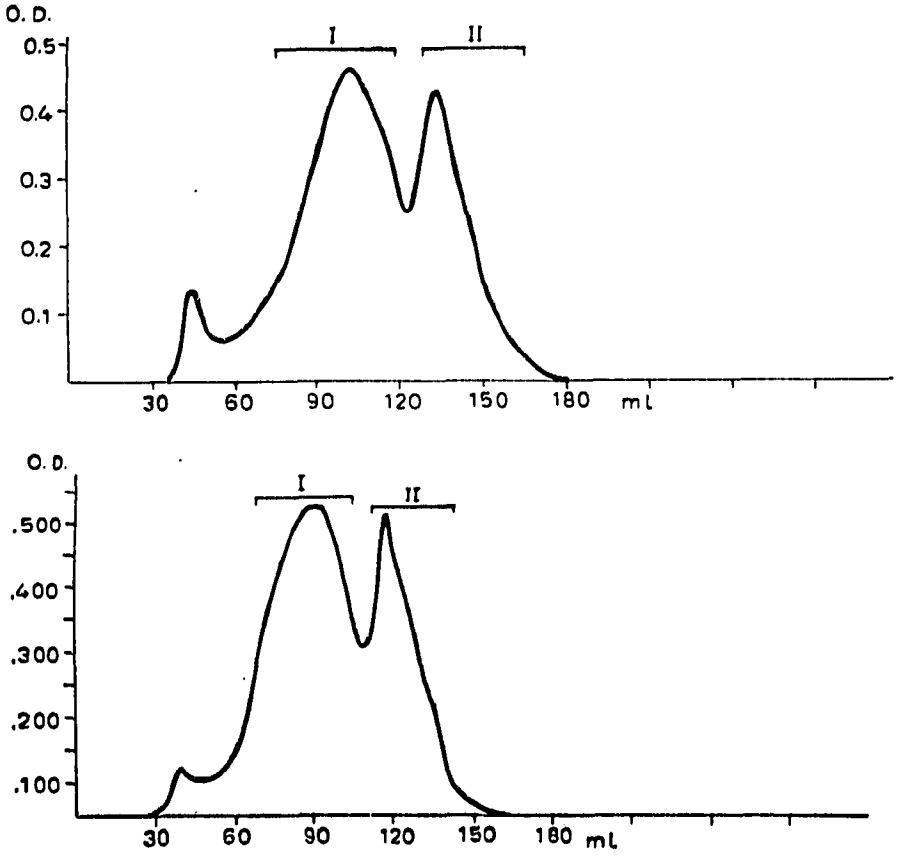

Fig. 3. Elution pattern of bovine chromogranine on a 2.5 × 33 cm Sepharose 4 B column at pH 7.4. The minor peak is eluted with the exclusion volume.

Fig. 4. Elution pattern on equine chromogranine on a 2.5 × 33 cm Sepharose 4 B column at pH 7.4.

of antigens and a single precipitation band was observed in all instances. When the purified apparently homogeneous chromogranine was applied on to a Sepharose column equilibrated with 0.05 *M* Tris–HCl buffer at pH 7.4, three distinct peaks were obtained (Figs. 3 and 4). A minor peak appeared with the exclusion volume and two others of approximately the same size emerged with elution volumes corresponding to molecular weights of 90,000 and 45,000, respectively. Behaviour characteristic of chromogranine was also observed on acrylamide gel electrophoresis at pH 9.6; a single, sharp band was observed when 10 μg of protein were applied to the gel; on increasing the protein concentration, however, (25, 50 and 75 μg) two or three distinct bands were observed (Figs. 5 and 6).

Discussion

As reported by other authors[1,2,5,6], chromogranine can be easily purified from the catecholamine storage vesicles of the adrenal medulla. The purification procedure used was the same for both the bovine and equine vesicle lysates and the purified chromogranine from both species showed similar behaviour on gel filtration and acrylamide gel electrophoresis. The protein which, after re-chromatography on DE-52 cellulose, appeared immunochemically homogeneous, was consistently resolved into two components of multiple molecular weights when placed on the Sepharose column.

The same kind of heterogeneity was observed on acrylamide gel electrophoresis when the protein concentration was increased. These results would indicate that an association–dissociation phenomenon concerning the fundamental monomer takes

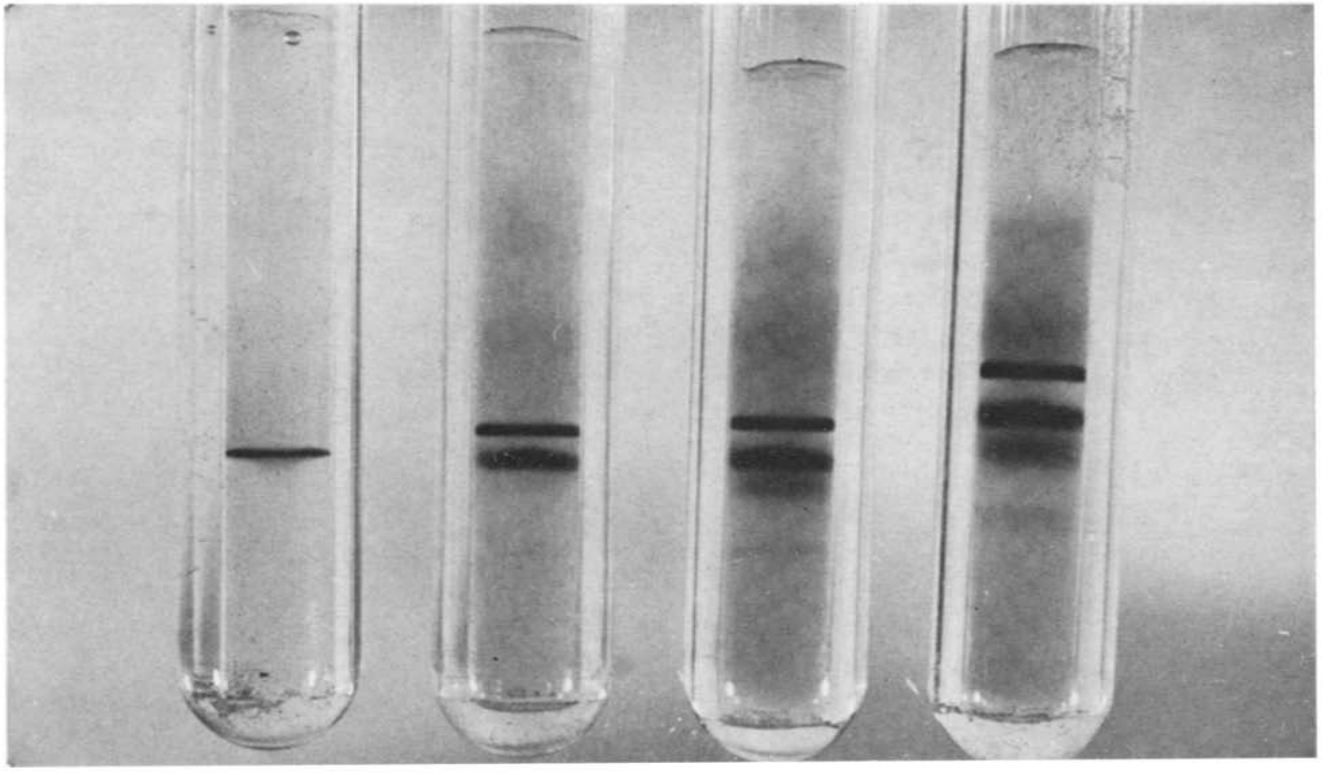

Fig. 5. Electrophoretic pattern of bovine chromogranine on acrylamide gel pH 9.6, increasing concentrations of the protein being used (from left to right: 10, 25, 50, and 75 μg).

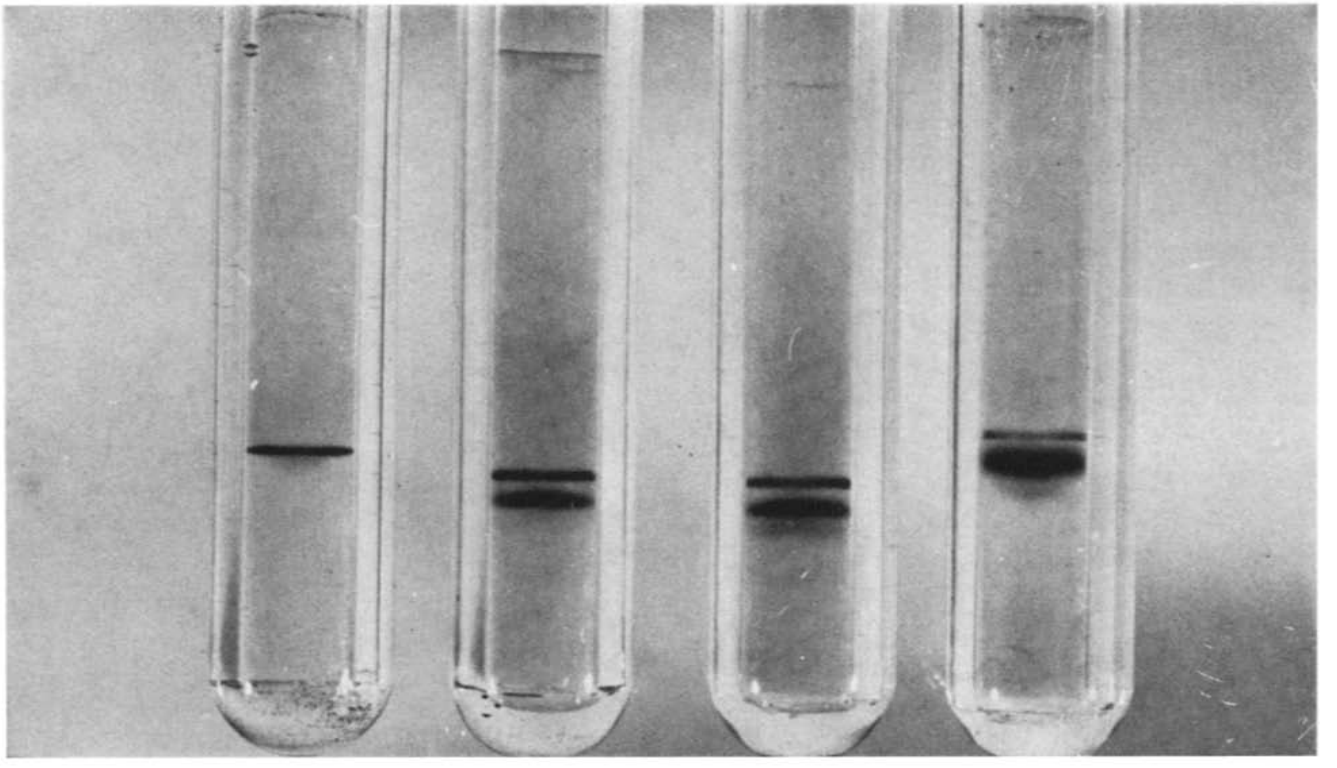

Fig. 6. Electrophoretic pattern of equine chromogranine on acrylamide gel pH 9.6, increasing protein concentrations being used (from left to right: 10, 25, 50, and 75 μg).

place under these experimental conditions; it remains to be seen whether or not this property of chromogranine has any significance with respect to its function within the catecholamine storage vesicles.

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