CHROM. 4637

Purification of α-amylase from C_3H mouse submaxillary gland by gel filtration

In previous studies on the marker-enzymes of the mouse submaxillary gland[1], these were compared to those of a spontaneous tumor derived from the submaxillary gland of a female mouse of the C_3H strain[2], and particular attention was given to proteolytic enzymes and amylase, which are partially maintained in the tumor tissue. It seemed interesting to purify these enzymes, in order to achieve a complete, comparative analysis of their chemico-physical and kinetic properties.

The present paper reports a simple procedure for the isolation of amylase from the submaxillary gland. The method is partially based on the fact that amylase is retarded when eluted through dextran gels, such as Sephadex. This property was already described by FLODIN[3] and by GELOTTE[9] for pancreatic α-amylase and by AURICCHIO[4,5] for an acid α-glucosidase from rat liver and human kidney.

Materials and methods

Adult male mice of the C_3H strain were sacrificed by cervical dislocation and the submaxillary glands promptly excised, freed of any connective tissue and frozen. Pools of 100 glands were homogenized in a Waring Blendor with 3 volumes of cold 5 m*M* Tris–HCl buffer, pH 7.4, and subsequently centrifuged at 15000 × *g* for 20 min in a refrigerated centrifuge.

The supernatant was recentrifuged at 100000 × *g* for 90 min in a Spinco Model L2-50 centrifuge. The sediment was discarded and protein concentration in the supernatant was estimated by the method of LOWRY[6], using bovine albumin as standard, while total amylase activity was determined according to the method of STREET AND CLOSE[7].

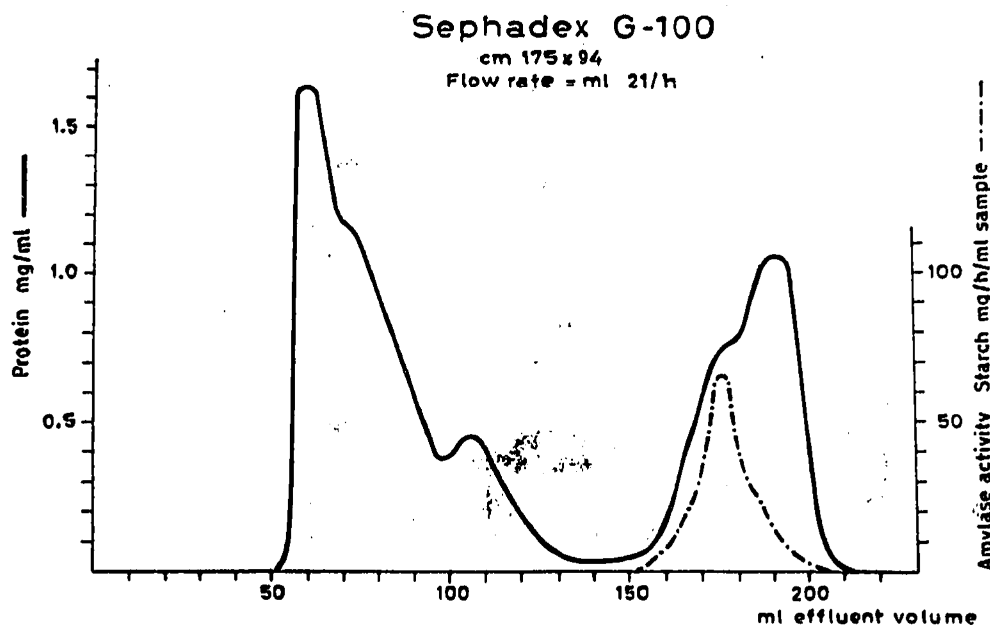

Fig. 1. Chromatography of a crude extract from male C_3H mouse submaxillary glands on Sephadex G-100.

Two ml of the supernatant (protein concentration: 75 mg/ml) were filtered through a Sephadex G-100 (Pharmacia, Uppsala, Lot. No. 9262) chromatographic column (1.75 × 94 cm), equilibrated and eluted with 5 m*M* Tris–HCl buffer, pH 7.4. Elution flow was kept constant by a peristaltic pump and all the operating procedures were made in a cold room.

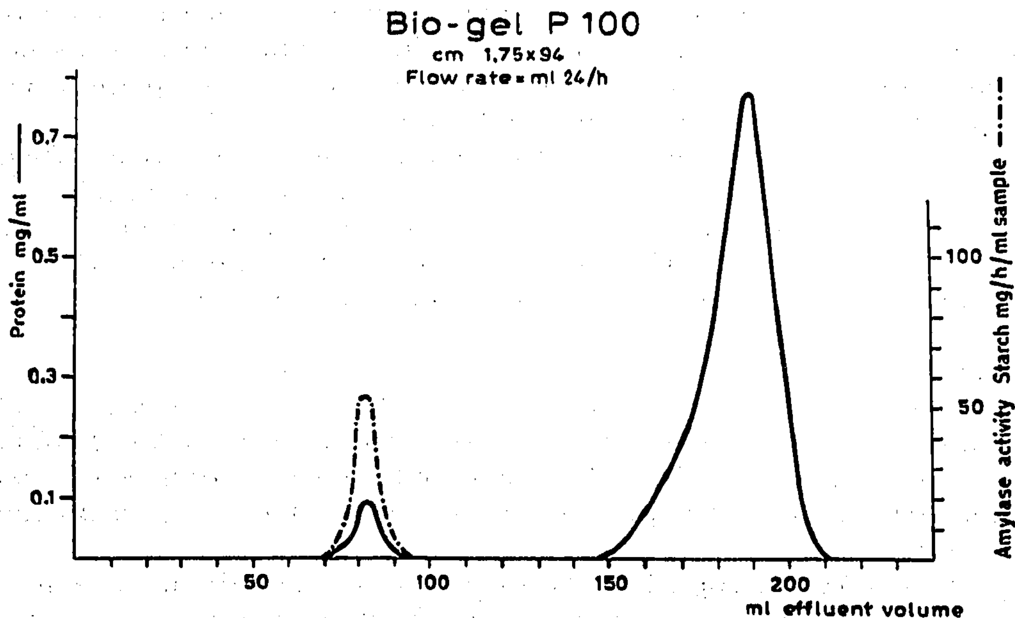

Fig. 2. Rechromatography on Bio-Gel P-100 of amylase pooled fractions eluted from Sephadex G-100.

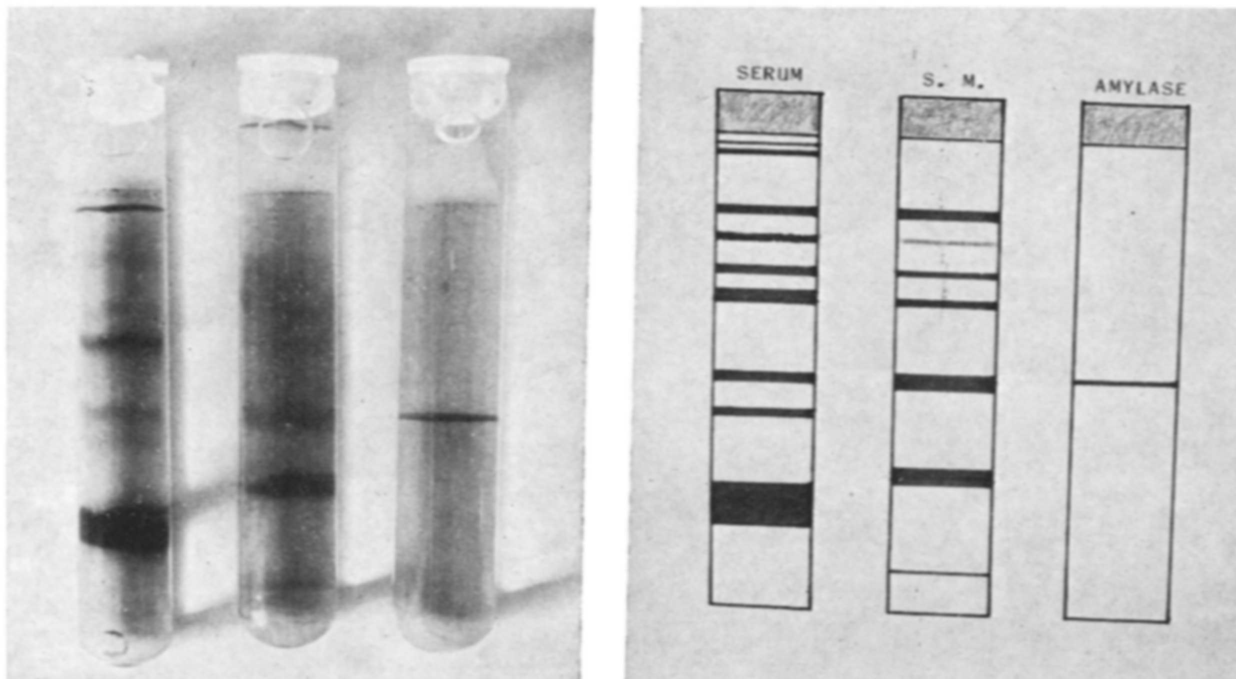

Fig. 3. Disc electrophoretic analysis of purified amylase (right), of male C_3H mouse submaxillary gland extract (middle), and of C_3H mouse serum (left).

The proteins were estimated directly on the column effluent by means of a LKB Uvicord at a wavelength of 254 mμ. Amylase activity was determined on the effluent fractions. Fractions containing amylase activity were pooled, dialysed overnight against cold distilled water, lyophilised, redissolved in a minimal volume of buffer and filtered through a Bio-Gel P-100 (Bio Rad, Richmond, Calif. Lot No. 2880) chromatographic column 1.75 × 94 cm), equilibrated and eluted as before.

Fractions containing amylase activity were pooled, dialysed overnight against cold distilled water, lyophilised and used for disc electrophoresis and ultracentrifuge analyses.

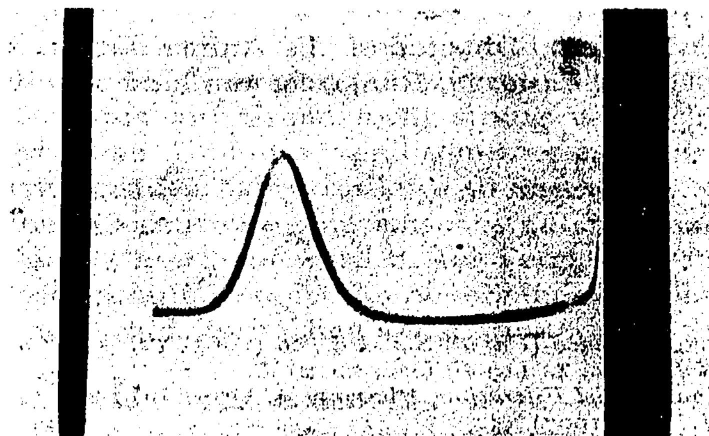

Fig. 4. Ultracentrifuge pattern of purified enzyme preparation. Protein concentration: 10 mg/ml; picture taken 4560 sec after reaching the full speed of 59 780 r.p.m.

Results

When the crude extract of submaxillary gland is filtered through a Sephadex G-100 column the amylase activity is eluted with the fourth protein peak, corresponding to a molecular weight of about 16000, as calculated according to the method of ANDREWS[8] (Fig. 1).

When the fractions containing amylase are refiltered through Bio Gel P-100, which is a molecular sieve very similar to Sephadex G-100, amylase activity is eluted earlier in a distinct and symmetrical peak, corresponding to a molecular weight of about 60000 (Fig. 2.).

TABLE I

PURIFICATION OF MOUSE SUBMAXILLARY GLAND AMYLASE

	ml	*Protein mg/ml*	*Total mg protein*	*Specific activity*[a]	*Total activity*[b]	*Yield %*
Submaxillary gland homogenate	2	75.0	150	12	1800	100
G-100 Amylase pool	42	0.42	17.6	92	1620	90
P-100 Amylase pool	20	0.06	1.2	1300	1560	87

[a] Specific activity = mg starch hydrolysed/h/mg sample at 37°.
[b] Total activity = amylase activity × total mg sample.

Electrophoretic analysis of this peak on polyacrylamide gel shows a single band corresponding to the α_1 proteins; the same band is also present in the serum of C_3H strain mice and in the crude extract of the submaxillary glands (Fig. 3). As observed in Fig. 4 a single symmetrical peak is also detectable in the ultracentrifuge pattern, with a sedimentation coefficient of $S^{1\%}_{20,w} = 2.24 \times 10^{-13}$.

Amylase was purified about 100 fold with a final yield of about 90 % of the starting activity (Table I) by the method described. It is noteworthy that when the Sephadex chromatography is performed after the Bio-Gel chromatography the recovery of the amylase activity is less. This phenomenon is probably due to the presence of a relevant amount of proteolytic enzymes, which are eluted together with amylase.

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