PREPARATION AND SOME PROPERTIES OF PORCINE CHYMOTRYPSINOGEN

M. T. Proskuryakov

Khimiya Prirodnykh Soedinenii, Vol. 3, No. 1, pp. 44–48, 1967

Trypsin and chymotrypsin are obtained by the method of Northrop, Kunitz, and Herriott [1] from the pancreatic glands of large horned cattle. It is not possible to isolate crystalline enzymes from pig glands by this method because of the specific differences of the tissues of these animals. However, it is known that the juice of the porcine pancreas has a high proteolytic activity [2].

In 1963, Travis and Liener [3], and also van Melle [4], obtained crystalline trypsin from an activated extract of porcine pancreatic gland. Ch'i Cheng-wu, et al. [5] isolated a number of crystalline enzymes possessing a chymotryptic and tryptic activity with respect to synthetic substrates, from porcine pancreatic glands. In Desnuelle's laboratory trypsin, chymotrypsin, and their precursors trypsinogen and chymotrypsinogen have been purified by chromatography on carboxymethylcellulose [6–8].

Table 1

Fractional Precipitation of a Sulfuric Acid Extract of Porcine Pancreas with Crystalline Ammonium Sulfate (calculated on 150 ml)

Degree of saturation with ammonium sulfate	Amount of protein in precipitate, mg	Activity of the precipitate, units × 10^3	Specific activity, units/mg	Percentage of the total activity of the precipitates
0.1	None	—	—	—
0.2	260	10.25	0.039	7.5
0.3	117	21.4	0.18	16
0.4	1000	100.0	0.1	74
0.5	124	3.0	0.024	2.2
0.6	32	None	—	—
0.7	None	—	—	—

The present paper gives the results of the isolation of porcine chymotrypsinogen and a study of some of its properties.

Experimental

After comminution, fresh porcine pancreatic glands (2 kg) were extracted with 0.25 N sulfuric acid. The clear extract was saturated with crystalline ammonium sulfate [9]. The dialyzed and lyophilized precipitate formed between 0.25 and 0.5 saturation was chromatographed on CM-cellulose [10].

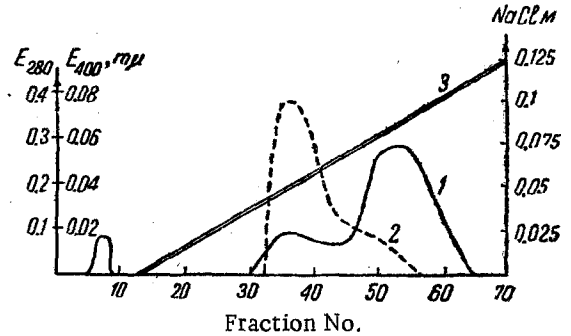

Fig. 1. Chromatographic curves of the precipitate salted out between 0.25 and 0.5 saturation on an analytical column in a concentration gradient of sodium chloride. 1) Protein; 2) activity; 3) concentration of sodium chloride.

Table 1 gives the results of the fractional salting out of an extract of the gland with ammonium sulfate. The activity of the chymotrypsinogen was determined after activation with trypsin (ratio of chymotrypsinogen to trypsin = 100:1 at pH 7.6, 0.1 M tris buffer, 20° C, 20 min) from the rate of hydrolysis of N-acetyl-DL-phenylalanine p-nitroanilide and was expressed in absorption units E_{400} after 1 hr at 37° C [11]. The amount of protein was determined from the absorption of the solutions at E_{280}. The absorption of a solution of chymotrypsinogen with a concentration of 1 mg/ml corresponded to a figure of 1.25.

As can be seen from Table 1, all the protein was salted out of the gland extract at 0.6 saturation. The precipitates between 0.2 and 0.5 saturation were richest in chymotrypsinogen;

here 90% of the whole chymotryptic activity of the extract was salted out.

It was established by chromatography on an analytical column with CM-cellulose in a concentration gradient of sodium chloride (Fig. 1) that the protein corresponding to the chymotrypsinogen peak issued from the column at a 0.05 M concentration of sodium chloride in the buffer. [Column of CM-cellulose (1 × 25 cm) equilibrated with 0.05 M sodium acetate buffer at pH 4.4; charged with 100 mg of protein in 2 ml of the same buffer; 12-ml fractions, 20 min].

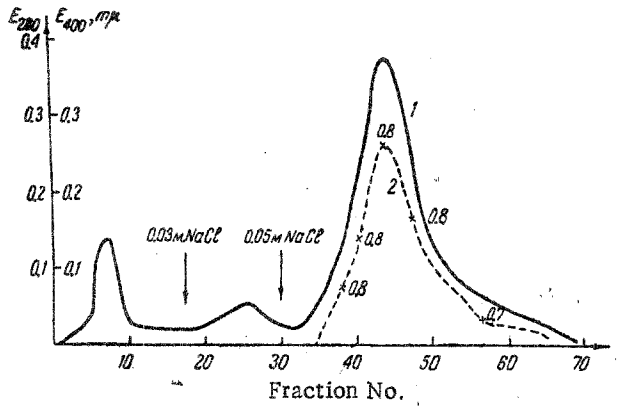

Fig. 2. Results of chromatographic purification of chymotrypsinogen on a preparative column. 1) Protein; 2) activity (the figures on the curve show the specific activity).

The enzyme was purified in preparative amounts on a column (3 × 12 cm) of CM-cellulose. The column was equilibrated with 0.05 M sodium acetate buffer at pH 4.4. It was charged with 1000 mg of protein in 10 ml of the same buffer. 35-ml fractions, 20 min (Fig. 2). All the operations up to the isolation and purification of the chymotrypsin (Table 2) were carried out in a cold room at 4–5° C. In determining the purity, the activity of the sulfuric acid extract was taken as the initial activity. Fractional precipitation raised the degree of purification of the preparation to 2.1. The chromatographically purified material had a degree of purity of 10.4. The yield of enzyme from 1 kg of gland was 1.6 g.

The homogeneity of the sample was established by paper electrophoresis (Fig. 3) in the free buffer solution. When the enzyme was rechromatographed on CM-cellulose, a single peak was obtained with an activity close to the initial activity. The isoelectric point was determined by free electrophoresis in an apparatus of the Microtiselius type in a 0.01 M sodium acetate buffer at pH 8.0–10.0. The lowest mobility towards the cathode was at pH 8.7 and towards the anode at pH 8.9. The isoelectric point was recorded at pH 8.8.

Table 2

Main Stages in the Purification of the Chymotrypsinogen
(Calculated on 2 kg of fresh gland)

Stage of purification	Weight or volume	Amount of protein, g	Activity in the precipitate, units × 10^3	Specific activity, units/mg	Yield (with respect to activity), %	Degree of purification
Extract	6 l	126	9.5	0.076	100	1
Precipitate from 0.2–0.5 saturation	30 g	45	7.2	0.16	75	2.1
Chromatographed sample	3.2 g	3.2	2.56	0.8	36	10.4

The stability of solutions of chymotrypsinogen was studied at 37° C and various pH values in Teorell and Stenhagen's universal buffer mixture. Solutions of chymotrypsinogen (0.07 mg/ml) at pH 6.0 and above showed no activity only 2 hr after activation with trypsin, while after 7 hr the activity in a solution of pH 1.0 fell to half, and it had disappeared at pH 5.0. After 24 hr exposure, only a solution with pH 3.0 was active. Consequently porcine chymotrypsin is most stable in solutions at pH 3.0.

The influence of the pH on the trypsin-activation of the enzyme was studied in universal buffer mixture with pH values from 2.0 to 12.0 at 20° C. The ratio of trypsin to chymotrypsinogen in these experiments was 10:1. The chymotrypsinogen was not activated at pH 4.0 and below or at pH 12.0. Activation took place most completely in solutions with pH 7.0–8.0. The optimum pH value for the activation of chymotrypsinogen with trypsin was 7.6.

Summary

A homogeneous preparation of chymotrypsinogen has been obtained by the fractional salting out of an acid extract of porcine pancreas and subsequent chromatography on a column of CM-cellulose. The isoelectric point of the enzyme has been found to be pH 8.8 and the optimum value of the pH for activation with trypsin 7.6. The enzyme is most stable in solutions with pH 3.0.

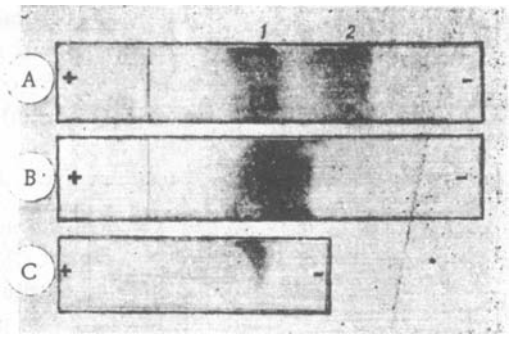

Fig. 3. Results of the electrophoresis of porcine chymotrypsinogen on paper. A) Electrophoregram of the material salted out between 0.2 and 0.5 saturation; B) veronal buffer with pH 8.6; C) citrate-phosphate buffer at pH 4.0. The chymotrypsinogen is the less mobile fraction.

REFERENCES

1. D. Northrop, M. Kunitz, and R. Herriott, Crystalline Enzymes [Russian translation], Moscow, 95, 1949.
2. A. V. Kvasnitskii, Physiology of Digestion in the Pig [in Russian], Moscow, 83, 1951.
3. J. Travis and I. Liener, Arch. Biochem. and Biophys., 97, 218, 1962.
4. P. J. van Melle, S. H. Samsa, and R. J. Westfall, Enzymologia, **26**, 133, 1963.
5. Ch'i Cheng-wu, Wu K'o-tso, and Hsü Shih-jung, RZhKhim Bkh., **24**, 32, 1964; C. A., **59**, 10391a, 1963.
6. S. Maroux, M. Rovery, and P. Desnuelle, Biochim. et Biophys. acta., **69**, 202, 1962.
7. M. Charles, M. Robery, A. Guidoni, and P. Desnuelle, Biochim. et Biophys. acta., **69**. 115, 1963.
8. M. Rovery, O. Guy, and P. Desnuelle, Biochim. et Biophys. acta., **42**, 554, 1960.
9. M. Dixon and E. C. Webb, Enzymes [Russian translation], Moscow, 52, 1961.
10. L. M. Ginodman, collection: Modern Methods in Biochemistry [in Russian], Moscow, 37, 1964.
11. H. Tuppy, U. Wiesberger, E. Wintersberger, Hoppe-Seyl. Z., **329**, 278, 1962.

15 November 1965

Institute of the Chemistry of Natural Compounds, AS USSR
Kuba Medical Institute