# FOWL TRYPSIN AND CHYMOTRYPSIN

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The pancreatic proteases of large horned cattle are among the well-studied enzymes [1]. Porcine trypsin has been obtained in the crystalline state and has been investigated [2, 3]. The trypsin and chymotrypsin of birds has been studied little. Two papers are known which give information on the isolation of turkey-cock trypsin and chick chymotrypsin [4] and also on the separation of fowl trypsin and chymotrypsin on diethylaminoethylcellulose [5].

The present paper gives the results of the purification of fowl pancreatic enzymes on carboxymethylcellulose and some properties of fowl trypsin and chymotrypsin.

As can be seen from the chromatogram (Fig. 1), all the protein deposited on the column was separated into six fractions. Fraction (V) possessed the milk-clotting activity that is characteristic of chymotrypsin. BAEE esterase activity, which is characteristic for trypsin, was possessed by fractions (II), (III), and (IV). Table 1 gives the quantitative results of chromatography which permit the conclusion that fraction (III) corresponds to trypsin and (V) to chymotrypsin.

The fractions were collected, dialyzed against water, sublimed, and studied. The individual enzymes were homogeneous on rechromatography, on electrophoresis on paper [10], and in a polyacrylamide gel [11], and also on gel chromatography in a thin layer of Sephadex G-75 [12]. However, the initial salted-out product, on paper electrophoresis (pH 8.6) separated distinctly into three zones, two of which (anionic and cationic) had proteolytic activity. Another anionic zone caused no proteolysis. In a polyacrylamide gel, four protein zones were found in the salted-out product.

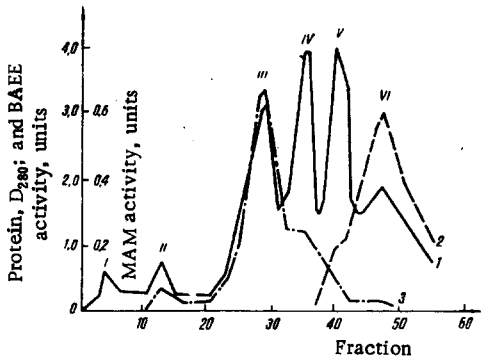


Fig. 1. Chromatogram of an activated salting-out product from fowl pancreatic gland: 1) protein, $D_{280}$; 2) MAM* activity, units; 3) BAEE† activity, units.

From the figures of Table 2, it is possible to judge the specific activities of fowl trypsin and chymotrypsin in comparison with the crystalline enzymes of large horned cattle. Attention is attracted by the high proteolytic activity of fowl trypsin and chymotrypsin. The specific activity of fowl chymotrypsin with respect to MAM (milk–acetate mixture) and of trypsin with respect to BAEE (N-benzoyl-DL-arginine ethyl ester) are somewhat lower than for the corresponding bovine enzymes.

The isoelectric point of the trypsin was found to be pH 10.6 and that of the chymotrypsin pH 8.0 from the electrophoretic mobility of the enzymes on paper in buffer solutions with various pH values [12]. Fowl trypsin had its optimum activity at pH 7.0-8.0 with respect to casein and hemoglobin, casein being hydrolyzed better than hemoglobin.

The heat stability of fowl chymotrypsin is somewhat higher than that of cattle chymotrypsin, and its optimum is found at pH 5.0. The range of pH values at which the fowl enzyme is stable is considerably wider.

The fowl enzymes behave identically to the cattle enzymes with respect to natural inhibitors. Kunitz's soya

* Milk–acetate mixture

† N-Benzoyl-DL-arginine ethyl ester

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TABLE 1. Quantitative Characteristics of the Chromatography of an Activated Salting-out Product from Fowl Pancreatic Gland

| Fraction | Protein, $D_{280}$ | Activity, units | | Specific activity, units/$D_{280}$ | |
|---|---|---|---|---|---|
| | | MAM | BAEE | MAM | BAEE |
| | deposited on the column | | | | |
| | 1650 | 165 | 361,7 | 0,1 | 0,22 |
| II | 45 | 0,9 | 10,35 | 0,02 | 0,23 |
| III | 279 | 11,16 | 306,0 | 0,04 | 1,10 |
| IV | 198 | 9,90 | 25,74 | 0,05 | 0,13 |
| V | 216 | 23,76 | 0 | 0,11 | — |
| VI | 297 | 100,98 | 24,76 | 0,34 | 0,08 |
| Total amt. in fractions | 1035 | 146,7 | 365,85 | — | — |

TABLE 2. Specific Activities of Chromatographically Purified Fowl Trypsin and Chymotrypsin in Comparison with the Analogous Enzymes of Large Horned Cattle

| Enzyme | Activity, units | | |
|---|---|---|---|
| | proteolytic | MAM | BAEE |
| Trysin | | | |
| fowl | 13,7 | — | 1,1 |
| bovine | 6,9 | — | 1,0 |
| Chymotrypsin | | | |
| fowl | 5,6 | 0,34 | — |
| bovine | 2,4 | 0,5 | — |

inhibitor in twofold molar excess suppresses the activity of fowl and bovine trypsins in veronal buffer with pH 8.2 containing 0.02 M calcium chloride at 35°C in 30 min. At the same time, a fivefold excess of the inhibitor only partially inhibited the chymotrypsins. Another natural inhibitor, Trasylol, has a similar effect on the enzymes of the animals compared.

By thin-layer chromatography in a layer of Sephadex G-75 gel [13] we found that the molecular weight of the fowl trypsin was 24,000 and that of the chymotrypsin 36,000. As the standard proteins we used cattle trypsin, chymotrypsin, chymotrypsinogen, ribonuclease, and hemoglobin.

## EXPERIMENTAL

Fresh fowl pancreatic glands were extracted with 0.1 N sulfuric acid (two volumes) for a day with periodic stirring. After the separation of the dense residue, crystalline ammonium sulfate was added to the extract to 0.2% saturation [6], and the pH was brought to 4.5 with 10 M caustic soda solution. Then the extract was filtered through a folded filter and was salted out with ammonium sulfate at 0.7 saturation. The precipitate was collected on a Buchner funnel, dissolved in water, and activated in the presence of 0.05 M calcium chloride at pH 8.0 with trypsin in an amount of 1 mg of trypsin per g of precipitate at 4-6°C.

The degree of activity of the chymotrypsin was determined from the rate of clotting of MAM by N. P. Pyatnitskii's method [7], that of trypsin from the hydrolysis of BAEE [8], and the proteolytic activity by Anson's method [9]. The concentration of protein was determined from the optical densities of the solutions in UV light, $D_{280}$. After activation, the solution of the salted-out product was dialyzed against water, filtered, and chromatographed on a column of carboxymethylcellulose. The column (3 × 12 cm), equilibrated with 0.05 M acetate buffer having pH 4.4, was charged with 1 g of the salted-out product, which was eluted with a gradient of increasing concentrations of sodium chloride in the initial buffer of from 0 to 0.3 M. The rate of elution was 1 ml/min and the fraction volume 12 ml. All the purification procedures were performed at 4-5°C.

## CONCLUSIONS

By chromatography on a carboxymethylcellulose column, trypsin and chymotrypsin have been obtained from fowl pancreatic gland. The molecular weights, isoelectric points, and optimum reactions of the media for activity and heat stability have been determined, and the behavior of these enzymes with respect to natural trypsin inhibitors has been investigated. A comparative study of the enzymes of the fowl and of large horned cattle has been performed.

## LITERATURE CITED


1. J. Northrop, M. Kunitz, and R. Herriott, Crystalline Enzymes, Columbia University Press (1948).
2. J. Travis and I. Liener, Arch. Biochem. and Biophys., 97, 218 (1962).
3. P. Melle, S. Samsa, and R. Westfall, Enzymologia, 26, 133 (1963).
4. C. Ryan, Arch. Biochem. and Biophys., 110, 169 (1965).

5. R. Zelikson, G. Eliam-Rubin, and R. Kulka, J. Biol. Chem., 246, 19, 6115 (1971).
6. Z. S. Bronovitskaya and V. P. Gortov, Prikl. Biokhim. i Mikrobiol., 3, No. 6, 707 (1967).
7. N. P. Pyatnitskii and M. N. Selyukova, Vopr. Pitaniya, 2, 42 (1960).
8. C. Figarella, J. Tauler, and H. Sarles, Bull. Soc. Chim. Biol., 4, 679 (1965).
9. M. Anson, J. Gen. Physiol., 22, 1, 79 (1938).
10. E. P. Smolichev, Tr. Stalinabadskogo Medinstituta, 21, No. 3, 70 (1956).
11. P. Reisfeld, U. Lewis, and D. Williams, Nature, 195, 4838, 281 (1962).
12. F. L. Kalinin, V. P. Lobov, and V. A. Zhidkov, Handbook of Biochemistry [in Russian], Kiev (1971), p. 892.
13. P. Andrews, Biochem. J., 91, No. 2, 222 (1964).