AN IMPROVED METHOD FOR THE CRYSTALLIZATION OF TRYPSIN

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The yields of crystalline trypsin prepared by the original method (1) are generally far below the values to be expected if all of the trypsinogen were changed into trypsin. The specific activity of the enzyme also tends to be low. This is due to a second reaction (2) which occurs simultaneously with the transformation of trypsinogen into trypsin—part of the trypsinogen is transformed into an “inert protein” which cannot be changed into trypsin by any known means. The formation of “inert protein” can be completely prevented (3), however, by allowing the autocatalytic conversion of trypsinogen to trypsin to proceed in the presence of calcium ions. Under these conditions, the trypsinogen is converted quantitatively into trypsin which can then be easily crystallized. Both the yield and quality of the crystalline trypsin are greatly improved.

The new method for the preparation of crystalline trypsin is as follows:—

1. *Conversion of Trypsinogen into Trypsin in the Presence of Calcium Chloride.*—Dissolve 30 gm. dried crystalline trypsinogen¹ (or 50 gm. semidry filter cake) in 200 ml. 0.005 M hydrochloric acid. Add the solution to a previously prepared ice-cold mixture of 100 ml. 1 M calcium chloride, 250 ml. 0.4 M² borate buffer, pH 8.0, and 400 ml. distilled water. Adjust the volume of the mixture with water to 1000 ml., and leave in cold room at about 5°C. for 18 to 24 hours.

2. *Removal of Calcium Ions.*—Add 2 gm. standard super cel (Johns-Manville Corporation) and filter in cold room with suction through soft paper (Eaton-Dikeman No. 303). Reject filter cake. Adjust filtrate to pH 3.0 (tested with methyl orange on spot plate) with 5 N sulfuric acid (about 4 ml.). Add solid ammonium sulfate (242 gm. per 1000 ml.) to 0.4 saturation and leave in cold room for 2 days. A heavy sediment of CaSO₄ crystals is formed. Filter with suction through soft paper (Whatman No. 3). Reject precipitate.

3. *Precipitation of Amorphous Trypsin.*—Add solid ammonium sulfate to

¹ The crystalline trypsinogen prepared by the method of Kunitz and Northrop generally contains about 50 per cent anhydrous MgSO₄.

² Stock borate solution contains 49.6 gm. boric acid and 80 ml. 5 N sodium hydroxide per 1000 ml. solution. 0.4 M borate buffers, pH 8.0 and 9.0, are mixtures of 100 parts stock borate and 78.6 and 17.6 parts 0.4 M hydrochloric acid respectively.

filtrate to 0.7 saturation (202 gm. per 1000 ml.) and filter with suction through hardened paper (Schleicher & Schüll No. 575). Reject filtrate. Dissolve filter cake (about 50 gm.) in 150 ml. distilled water and reprecipitate the trypsin by the *slow* addition, while stirring, of 350 ml. saturated ammonium sulfate from a dropping funnel. Filter with suction through hardened paper (S. & S. No. 575), size 18.5 cm. or larger, and wash filter cake, as described by Kunitz and Northrop (1), with saturated magnesium sulfate in 0.02 N sulfuric acid to remove excess ammonium sulfate. Yield about 50 gm.

4. *Crystallization of Trypsin*.—Dissolve semidry filter cake in ice-cold 0.4 M borate buffer, pH 9.0 (10 ml. per 10 gm. filter cake), at 2–5°C. (in an ice water bath). Crystals of fine needles appear almost immediately. Leave in cold room for 24 hours, then filter (cold) with suction on hardened paper (S. & S. No. 575). Wash the crystals several times with cold 0.5 saturated magnesium sulfate in 0.1 M borate buffer, pH 8.0, and then, at room temperature, with saturated magnesium sulfate in 0.1 N sulfuric acid. Yield 15 to 20 gm.

5. *Recrystallization of Trypsin*.—Dissolve the semidry filter cake in 0.02 N sulfuric acid (10 ml. per 10 gm. filter cake), mixing the filter cake gradually into the acid to avoid foam. Filter clear on small fluted No. 3 Whatman paper. Wash paper with several milliliters of 0.02 N H₂SO₄. Cool filtrate to about 5°C. and then adjust to pH 8.0 (tested with cresol red on spot plate) with cold 0.4 M borate buffer, pH 9.0 (about 8 ml. per 15 ml. solution). Crystallization begins almost immediately. Leave in cold room for 24 hours, then filter and wash as described for the first crystallization. Yield 10 to 15 gm. Dry filter cake for several days in a dry refrigerator, grind to fine powder, and store in cold room.

BIBLIOGRAPHY

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