contains trypsinogen, the inactive form of trypsin, and becomes active upon the addition of enterokinase or upon dissolving in concentrated magnesium or ammonium sulfate.[2] Under the latter conditions the activation is autocatalytic. This trypsinogen has now been isolated in crystalline form as short triangular prisms. It is a protein and has no proteolytic activity but becomes active under the same conditions as does the original filtrate from the chymo-trypsinogen. Thus, on standing in concentrated magnesium sulfate solutions at pH 7.0–8.0 this protein is transformed into the active proteolytic enzyme, trypsin, which may then be crystallized in the form of short rectangular prisms or fine needles. The activation reaction is autocatalytic. The activation curve for the crude trypsinogen solution shows a prolonged lag period due probably to the presence of an inhibiting substance. This prolonged lag period allows crystallization of trypsinogen to take place before activation. The solutions of crystalline trypsinogen, however, activate much more rapidly and there is no lag period. For this reason it has not been possible, so far, to recrystallize the trypsinogen, since the conditions for crystallization are also those for activation. The trypsinogen is, therefore, transformed to active trypsin before trypsinogen crystals can form, and the crystals which later appear are those of active trypsin instead of trypsinogen. Crystalline trypsin obtained in this way is identical, so far as has been determined, with the crystalline trypsin previously isolated from active pancreatic extract.[3]

The method of isolation of these crystalline proteins is briefly as follows. All the solutions used must be cooled to about 5° C. and all operations are carried out in the icebox. The mother liquor from the chymotrypsinogen crystallization is titrated to pH 4.0 with 2.5 M sulfuric acid, brought to 0.7 saturated ammonium sulfate and filtered. 100 gm of the precipitate is dissolved in 300 ml water, brought to 0.4 saturated ammonium sulfate and filtered. The filtrate is brought to 0.6 saturated ammonium sulfate by slow addition of saturated ammonium sulfate and filtered with suction. The precipitate is washed twice on the filter with saturated magnesium sulfate. Ten gm of filter cake is dissolved in 10 ml 0.4 M borate buffer pH 9.0; 17 ml saturated magnesium sulfate is added and the solution allowed to stand at 6° C. Short triangular pyramids appear in the course of 2 to 3 days. If the solution is inoculated crystallization is much more rapid, but the crystals are not so well formed. Occasionally the solutions become active and crystallization stops or crystals of the active trypsin may appear.

[2] M. Kunitz and J. H. Northrop, SCIENCE, 80: 190, 1934.

[3] M. Kunitz and J. H. Northrop, *Jour. Gen. Physiol.* (in press).

CONVERSION OF TRYPSINOGEN TO TRYPSIN AND CRYSTALLIZATION OF TRYPSIN

Trypsinogen crystals are washed with 0.5 saturated magnesium sulfate in 0.10 M borate buffer pH 8.0 and then with saturated magnesium sulfate in 0.1 M acetic acid. Ten gm filter cake is suspended in 5 ml 0.01 M sulfuric acid and 2.5 M sulfuric acid added drop by drop until the crystals dissolve. Ten ml saturated magnesium sulfate and 5 ml 0.4 M borate buffer pH 9.0 is added and pH adjusted with saturated potassium bicarbonate solution to pink to phenol red on test plate. The solution is inoculated and allowed to stand at about 5° C. A heavy crop of trypsin crystals forms in a few hours. The first crystals may be poorly defined. Recrystallization is carried out in the same way but with slightly more dilute solution of the protein. The crystals are needle-shaped and may be quite short or may appear in rosettes. The purified trypsin obtained from active cattle pancreas, as previously described,[3] may be crystallized under the same conditions. Much better crystals are obtained in this way than when crystallization is carried out at pH 4.0 and room temperature with ammonium sulfate, as in the original method.

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