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# On the Electrophoretic Heterogeneity of Trypsin

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## Introduction

In previous studies (1) it has been shown that crystalline trypsin is not a homogeneous protein, but that in the presence of calcium ions at pH 4.7 it contains two electrophoretically distinct components (with mobilities of $5.35 \times 10^{-5}$ and $4.76 \times 10^{-5}$ sq. cm./v./sec.) both of which appear to be proteolytically active. This electrophoretic heterogeneity is evident only in the presence of calcium and certain other ions, in the absence of which the enzyme appears to be homogeneous due to molecular interaction. This finding was also confirmed with the inactive isopropyl phosphorofluoridate (DFP) trypsin derivative (2). In this case, the inactivation of the enzyme prevents the molecular interaction. In the present communication we wish to report the successful isolation of one of the components of trypsin in an electrophoretically pure state and the comparison of the specific proteolytic activities of the separated component and the original enzyme.

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## Experimental and Results

The separation of the rapidly migrating trypsin component (representing about 65% of the enzyme) was accomplished in the 11-ml. separation cell of the Klett-Tiselius electrophoresis apparatus by subjecting a 2% solution of trypsin to electrophoretic migration for 11 hr. with back compensation. The experiments were carried out at 2°C. and a field strength of 4 v./cm. in a pH 4.7 calcium acetate buffer of 0.1 ionic strength.

In Fig. 1 are presented the electrophoretic diagrams of the original trypsin solution and of the faster component isolated in an electrophoret-

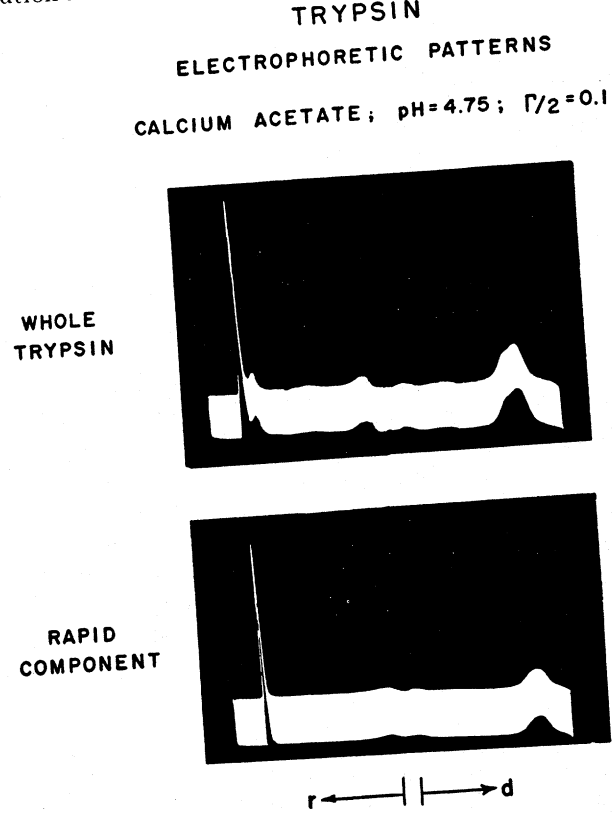


Fig. 1. Electrophoretic diagrams of trypsin preparations. *a*: Original whole trypsin. *b*: Rapid component after isolation in Klett separation cell. Ca Acetate buffer, pH = 4.7; Γ/2 = 0.1; 1800 sec. at 12 v./cm.

TABLE I

*Relative Enzymatic Activities of Trypsin Fractions*

| Fraction | Substrate | | |
|---|---|---|---|
| | Hemoglobin | BAEE[a] | TSAEE[b] |
| Crystalline trypsin | 1.00 | 1.00 | 1.00 |
| Rapid (run I) | — | — | 1.14 |
| Rapid (run II) | — | — | 0.91 |
| Rapid (run III) | 0.89 | 1.07 | — |
| Slow (enriched) | — | — | 0.97 |
| "Immobile" | — | — | 0.25 |

[a] Benzoylarginine ethyl ester.
[b] *p*-Toluenesulfonyl ethyl ester.

ically pure state from the rising limb of the separation cell. These analyses were carried out in a Perkin-Elmer Tiselius electrophoresis apparatus under conditions similar to those of the original separation. No comparable diagrams of the slower components were obtained as too little protein could be recovered. Furthermore, as was evident in the course of the separation runs, the slow component in the descending limb was highly diffuse and became disturbed in every separation run during back compensation. Nevertheless, the fraction which was supposed to be enriched with respect to the slow component was also analyzed with respect to its proteolytic activity, "slow (enriched)" in Table I. In a special run without back compensation, a sample of an electrophoretic component of even lower mobility, constituting *ca.* 1% of the total protein, was separated and assayed. This material is designated as "immobile" in Table I. In the evaluation of the data, it has to be kept in mind that any inactive proteolytic breakdown products of trypsin self-digestion would most probably be incorporated in the two slow fractions due to their very low electrophoretic mobility (3).

In Table I the proteolytic activities are presented for the original trypsin preparation and the fractions obtained. The data show clearly that the rapid component and the original trypsin have comparable proteolytic activities toward all substrates tried, i.e., toward urea-denatured hemoglobin, benzoylarginine ethyl ester, and *p*-toluenesulfonyl ethyl ester. The data presented seem to indicate that the tryptic activity of crystalline trypsin is not associated with only one of its principal electrophoretic components but that it is associated equally with both.

## Summary

Two electrophoretic components of trypsin were separated and shown to possess comparable proteolytic activity on natural and synthetic substrates.

## References

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