RESOLUTION OF α AND β ANHYDROTRYPSIN BY AFFINITY CHROMATOGRAPHY

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SUMMARY: pH gradient elution of anhydrotrypsin from a soybean inhibitor (Kunitz) affinity column provides good resolution of the α and β components. The usual method of preparation has been to elute discontinuously with a solution sufficiently acidic to remove all column-bound material in a single band. The fact that α and β anhydrotrypsins have significantly different affinities for immobilized soybean inhibitor means that these components must be considered separately in further work.

Shaw has shown that commercial trypsin preparations are composed chiefly of two active forms: the lys 131-ser 132 peptide bond is intact in β, and cleaved in α trypsin (1). Significant differences in the characteristics of α and β have been shown to exist, and it is now customary to work with the separated components (2,3). The α and β forms can be separated by Shaw's method (1), by pH gradient elution from an ovomucoid affinity column (4), or by salt gradient elution from SP Sephadex (3). The anhydro derivative prepared by Ako et al. (5) is usually made from commercial trypsin, and it therefore appeared likely that such anhydrotrypsin would be a mixture of α and β forms.

A soybean trypsin inhibitor (Kunitz, STI) affinity column was prepared by activating Sepharose 2B with cyanogen bromide and subsequent coupling with STI as described by Cuatrecasas et al. (6). The trypsin sample was applied to a 1 x 30 cm column equilibrated with 0.01 M citrate, 0.10 M KCl, 0.01 M $CaCl_2$. The resultant pH gradient is very nearly linear in the region of interest. Similar results were obtained with Agarose A-5-M (Biorad) as the support material. Higher flow rates were possible, and the column appeared to be more stable with repeated use; components eluted at somewhat lower pH than was effective with

Sepharose 2B. The bound trypsin capacity was about 1 mg/ml gel.

We have found component elution volumes under specified gradient conditions to be easily reproduced. Figure 1A is a typical chromatogram obtained with commercial trypsin. In Figure 1B two chromatograms are shown, one obtained with α trypsin, and the other with β trypsin as sample (these were prepared by salt gradient elution from SP Sephadex (3)). 1A and 1B show that α and β trypsin are clearly resolved and identified, similar to the results reported by Robinson et al. (4) with

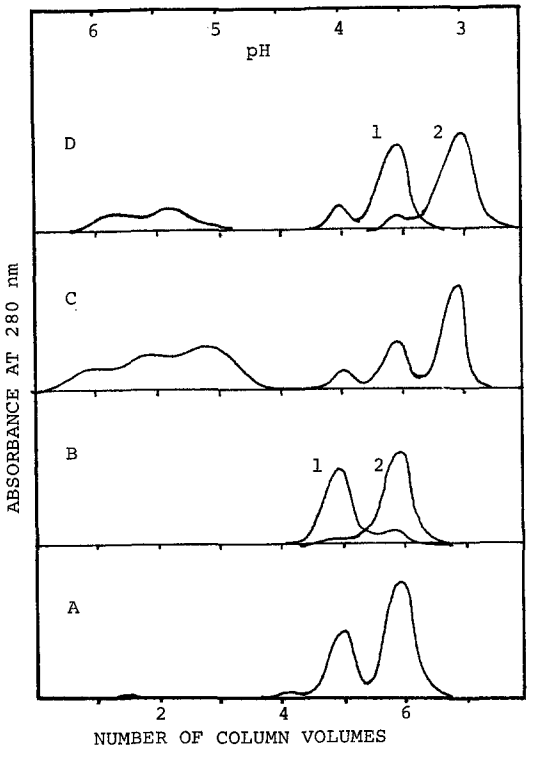

Figure 1. Chromatograms obtained by gradient elution of a 1x30 cm STI-Sepharose 2B affinity column. The fall-through of non-bound protein is not shown; the record starts with the gradient. Chromatograms of, A, commercial trypsin, B-1 and B-2, α and β trypsin, respectively, C, anhydrotrypsin prepared from commercial trypsin, D-1 and D-2, anhydrotrypsin prepared from α and β trypsin, respectively.

an ovomucoid affinity column. Figure 1C is a chromatogram of anhydrotrypsin prepared from commercial trypsin. Figure 1D shows chromatograms obtained separately with anhydrotrypsin samples prepared from α and from β trypsin. Comparison of Figures 1C and 1D identifies the α and β anhydrotrypsin components in the preparation begun with commercial trypsin. Comparison of 1B and 1D shows that α anhydrotrypsin and β trypsin elute in the same volumes. Chromatography of anhydrotrypsin prepared from commercial trypsin will therefore yield pure β anhydro, but the α anhydro derivative may contain small amounts of β trypsin. Pure α anhydrotrypsin can be prepared by starting with α trypsin, or by treating the anhydrotrypsin mixture with Nα tosyl lysyl chloroketone (TLCK) before chromatography. Figure 1C shows that an appreciable amount of material other than α and β anhydrotrypsin is bound to the column. Chromatography of phenyl methane sulfonyl trypsin (PMS trypsin) shows very little column-bindable material of any kind; subsequent hydrolysis and chromatography of this PMS trypsin also produces the early eluting components of Figure 1C, demonstrating that they are formed by hydrolysis of the PMS trypsin preparation. These components (there are at least two) which can complex with the immobilized STI but which are clearly neither α nor β anhydrotrypsin, may arise from the reaction of additional tryptic serine residues (7).

The fact that α and β anhydrotrypsin are resolved by an STI affinity column is itself evidence that their properties are significantly different, at least with respect to complex formation with STI. It is interesting that the anhydro modification improves the affinities of both α and β trypsin for the immobilized inhibitor, with the relative α-β affinities preserved. The gradient elution provides an easy method for the resolution of the α and β anhydrotrypsins, and it appears advisable to treat the α and β forms separately in further work.

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