By employing this simplified method, sugar solutions to be analyzed by ion-exchange chromatography can be prepared in a short time, which is a great advantage over the existing methods. Other advantages are the smaller possibilities for the loss of sugars and hydrolysis of sucrose during the preparation of sugar solutions. The chromatographic elution pattern of sugars obtained by this method is, as far as the plant materials we have thus far tested are concerned, essentially the same as those obtained by the standard method.

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[1] J. N. KHYM AND L. P. ZILL, *J. Am. Chem. Soc.*, 74 (1952) 2090.
[2] G. R. NOGGLE AND L. P. ZILL, *Arch. Biochem. Biophys.*, 41 (1952) 21.
[3] T. FUKUI AND Z. NIKUNI, *Nippon Nôgei-Kagaku Kaishi*, in the press.
[4] K. MORI AND M. NAKAMURA, *Bull. Agr. Chem. Soc. Japan*, in the press.
[5] H. G. PONTIS, E. CABIB AND L. F. LELOIR, *Biochim. Biophys. Acta*, 26 (1957) 146.
[6] M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.
[7] J. H. ROE, *J. Biol. Chem.*, 107 (1934) 15.

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Changes in the ultraviolet absorption spectrum of trypsin associated with disruption of tertiary structure

Changes in the ultraviolet absorption spectra of several proteins have been demonstrated when these were exposed to a variety of experimental conditions. The changes may arise from the ionization of the tyrosine hydroxyl groups[1,2,3] or from the disruption of hydrogen bonds involving tyrosine hydroxyl groups[4,5]. It has also been suggested that a change in charge of an ionizing group neighbouring a non-ionizable chromophoric group (*e.g.* tryptophan) may result in a shift in the absorption spectrum of the latter to lower wavelengths[6]. Recently, CHERVENKA has shown that the activation of chymotrypsinogen is accompanied by a shift in the absorption of the tyrosine residues to lower wavelengths[7]. He also demonstrated a spectral change accompanying the autolysis of chymotrypsin similar to that which occurred during urea treatment of either the zymogen or the enzyme. Under both conditions of denaturation, the difference spectrum resulted from a wavelength shift in the absorption of tryptophan and probably of all the chromophoric amino acid residues.

We have been unable to demonstrate a significant change in absorption during the activation of trypsinogen, but have observed a characteristic and reproducible difference spectrum when trypsin was autolysed in the absence of calcium or upon treatment with urea. These spectra show a striking similarity to those obtained by CHERVENKA with the chymotrypsin system.

In all experiments at pH 8.00, the reference cuvette of a Beckman DK-1 re-

cording spectrophotometer contained 1.81 mg trypsin/ml in 0.1 *M* tris(hydroxymethyl)aminomethane, 0.05 *M* $CaCl_2$. The test cuvette contained 3.5 ml of the experimental solution and, at zero time, 0.1 ml of 6.50% trypsin solution was added with a rapid mixing device designed by G. H. Dixon of this laboratory. Measurements of the absorption difference at 293 mμ were begun within 10 sec and difference spectra from 330 mμ to 240 mμ were taken at suitable intervals. The temperature was maintained at 25 ± 0.2° throughout.

The decrease in absorption of trypsin at 293 mμ in 0.1 *M* tris(hydroxymethyl)-aminomethane, pH 8.00 in the absence of calcium is shown in Fig. 1 together with a similar experiment in the presence of 0.01 *M* $CaCl_2$. Since the autolysis of trypsin in the absence of calcium has been amply demonstrated[8,9], there seems little doubt that this process is accompanied by a characteristic spectral change (Fig. 2).

The effects of 2 *M* and 8 *M* urea at pH 8.00 on the absorption of trypsin at 293 mμ are shown in Fig. 3. In the absence of calcium (Fig. 3A), the spectral change

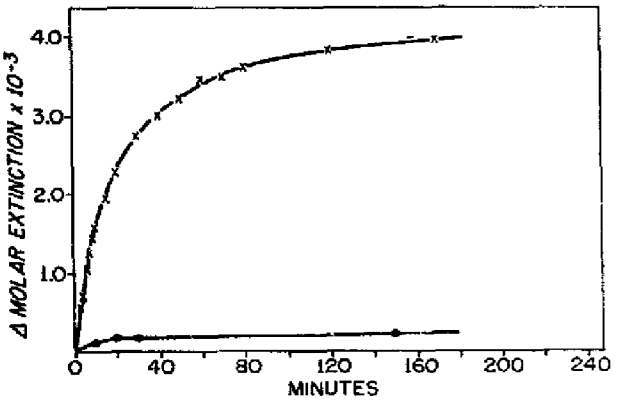

Fig. 1.

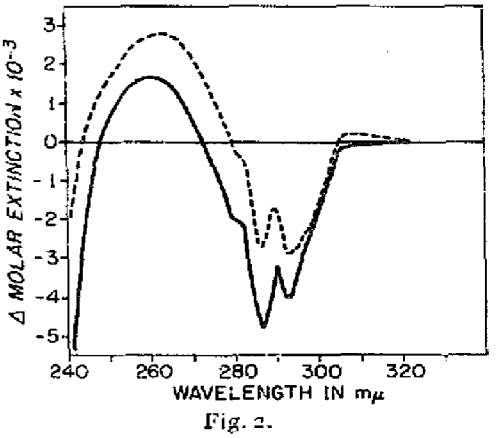

Fig. 2.

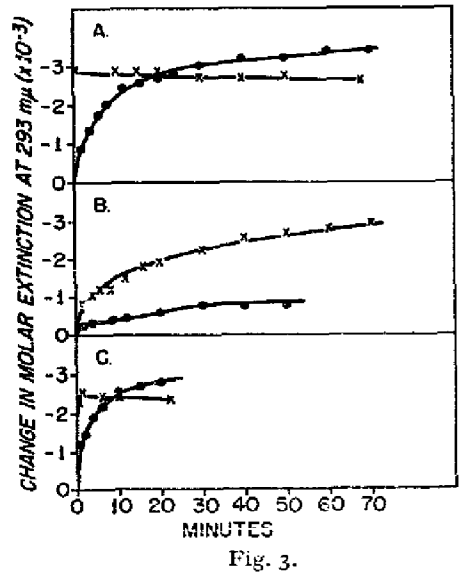

Fig. 3.

Fig. 1. Change in absorption at 293 mμ of trypsin during autolysis at 25°: ×——× trypsin in 0.1 *M* tris(hydroxymethyl)aminomethane, pH 8.00, no calcium: ●——● trypsin in 0.01 *M* $CaCl_2$, 0.1 *M* tris(hydroxymethyl)-aminomethane, pH 8.00.

Fig. 2. Difference spectra of trypsin solutions at 25°: ——— after 170-min autolysis in 0.1 *M* tris(hydroxymethyl)aminomethane, pH 8.00, no calcium; – – – – – after 5 min in 8 *M* urea, 0.1 *M* tris(hydroxymethyl)aminomethane, pH 8.00.

Fig. 3. Changes in absorption at 293 mμ of trypsin in 8 *M* (×——×) and 2 *M* (●——●) urea. A. Trypsin in urea, 0.1 *M* tris(hydroxymethyl)aminomethane, pH 8.00, no calcium. B. Trypsin in urea, 0.1 *M* tris(hydroxymethyl)-aminomethane, 0.05 *M* $CaCl_2$, pH 8.00. C. Trypsin in urea, pH 3.00.

is instantaneous in 8 *M* urea, but proceeds at measureable rates at lower concentrations. The presence of calcium (Fig. 3B) markedly reduces the rate of the spectral change. Similar effects of calcium on the chymotrypsin system were observed by CHERVENKA[7]. The spectral changes at pH 8.00 in solutions of urea less concentrated than 8 *M* probably result from both the effects of urea and of autolysis since trypsin is known to be autolysed at lower concentrations of urea even in the presence of calcium[10].

In another series of experiments the effects of urea on trypsin at pH 3.00 were followed (Fig. 3C). In these experiments the reference cell of the spectrophotometer contained 1.81 mg trypsin/ml in 0.001 *M* HCl. It is of interest that at each urea concentration the rates were greater than at pH 8.00 in the absence of calcium.

The effects of several acidic solutions on the absorption spectrum of trypsin were studied with trypsin at pH 8.00 as the reference. The changes in molar extinction at 293 mμ in solutions of pH 5.0, 3.0, and 1.0 were 0.1, 0.8, and 1.3, respectively. These changes were instantaneous and at pH 3.0 were not affected by the presence of calcium. The data are inadequate to ascribe the changes to a dependence on the ionization of carboxyl groups as has been done for lysozyme[8].

In a preliminary experiment the difference spectrum of trypsinogen in 7.5 *M* urea was found to be similar to that of trypsin in urea. These spectral changes do not involve the active center of trypsin, since no change in absorbancy was observed during the activation of trypsinogen or during reaction of trypsin with diisopropylfluorophosphate. Further studies on the factors affecting the rates and magnitudes of these spectral changes are contemplated.

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1 D. SHUGAR, *Biochem. J.*, 52 (1952) 142.
2 C. TANFORD, J. D. HAUENSTEIN AND D. G. RANDS, *J. Am. Chem. Soc.*, 77 (1955) 6409.
3 P. E. WILCOX, *Federation Proc.*, 16 (1957) 270.
4 H. A. SCHERAGA, *Biochim. Biophys. Acta*, 23 (1957) 196.
5 M. LASKOWSKI, JR., J. M. WIDOM, M. L. MCFADDEN AND H. A. SCHERAGA, *Biochim. Biophys. Acta*, 19 (1956) 581.
6 J. W. DONOVAN, M. LASKOWSKI, JR. AND H. A. SCHERAGA, *Biochim. Biophys. Acta*, 29 (1958) 455.
7 C. F. CHERVENKA, *Biochim. Biophys. Acta*, 31 (1959) 85.
8 L. GORINI, *Biochim. Biophys. Acta*, 7 (1951) 318.
9 L. W. CUNNINGHAM, JR., F. TIETZE, N. M. GREEN AND H. NEURATH, *Discussions Faraday Soc.*, 13 (1952) 58.
10 T. VISWANATHA AND I. E. LIENER, *J. Biol. Chem.*, 215 (1955) 777.

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