## Change of helical configuration in the activation of chymotrypsinogen

Since it has been demonstrated by DESNUELLE *et al.*[1] and NEURATH *et al.*[2] that trypsinogen and chymotrypsinogen are activated only through the cleavage of one peptide linkage, it is interesting to investigate the intramolecular changes of configuration accompanying this activation process. In fact, NEURATH *et al.*[3] found that the levorotation decreased markedly during this process. They thus came to a conclusion that the degree of helical formation was increased by the activation, giving rise to a configuration suitable for forming the active center.

Ingenious as it appears, this work of NEURATH *et al.* involves some serious uncertainties in drawing decisive conclusions. First of all, they determined only the specific rotation and not the rotatory disperion. As has been pointed out by one of the authors, denaturation of proteins into the corresponding $\beta$-forms is accompanied by a rise in the specific rotation[4]. Therefore, the decrease of levorotation does not necessarily reflect the increase of the helical fraction. Moreover, the presence of liberated peptides, especially in the case of trypsinogen, might also have contributed to the change of the specific rotation accompanying the reaction.

To exclude these ambiguous points, we compared the rotatory dispersion of chymotrypsinogen with that of $\pi$-chymotrypsin which was isolated from the former in a pure state. Chymotrypsinogen was prepared from beef pancreas according to the method of KUNITZ AND NORTHROP[5]. After being recrystallized several times in the presence of $10^{-3}$ *M* di-isopropylfluorophosphate and lyophylized, the sample showed less than 0.05 % of chymotrypsin activity and 0.05 % trypsin activity. $\pi$-chymostrypsin was prepared by activation (at 0° and pH 7.7) of the chymotrypsinogen with 1/50th its weight of trypsin in the presence of $\beta$-phenylpropionic acid. After complete activation, the mixture was dialysed against an HCl solution of pH 3 and lyophilized. The specific activity of this sample was 4.5 when measured with L-acetyltyrosine ethyl ester as substrate. Good homogeneity of chymotrypsinogen and $\pi$-chymotrypsin prepared was ascertained also by the patterns of their elution chromatograms.

Before carrying out the examination of rotation the existence of the $\beta$-configuration in the samples was checked by measuring the infrared absorption spectrum at 1910 $cm^{-1}$ which corresponds to the >C=O stretching frequency of the $\beta$-configuration[6]. With both samples the results were negative so that the existence of $\beta$-fraction could be left out of consideration. In the absence of $\beta$-configuration, the data of rotatory dispersion of proteins can be expressed by the following empirical formula[4]

$$\frac{\lambda^2-\lambda^2_0}{\lambda^2_0}[m'] = a_0^R + f^H a_0^H + \frac{\lambda^2_0}{\lambda^2-\lambda^2_0} f^H b_0^H \qquad (1)$$

where $\lambda$ and $\lambda_0$ are wavelength and characteristic wavelength, respectively, $a_0^R$ the intrinsic residue rotation, $[m']$ the effective residue rotation, $f^H$ the fraction of the helical part, and $a_0^H$ and $b_0^H$ are contributions of helical configuration, which have been determined to be +680 and −600, respectively, from the data of synthetic polypeptides. Effective residue rotation $[m']$ can be obtained from the specific rotation $[\alpha]$ by the equation:

$$[m'] = \frac{M}{100}\frac{3}{n^2+2}[\alpha] \qquad (2)$$

where M denotes the mean residue weight and $n$ the refractive index of the solvent.

In Fig. 1 are shown the rotatory dispersions measured for both samples with the assumption that $\lambda_0$ is 212 m$\mu$. Since $a_0^R$ of chymotrypsinogen and $\pi$-chymotrypsin has been found from the dispersion in 8 $M$ urea (in which $f^H = 0$) to be $-580$, the helical content $f^H$ could be determined from the slopes and intercepts of the curves

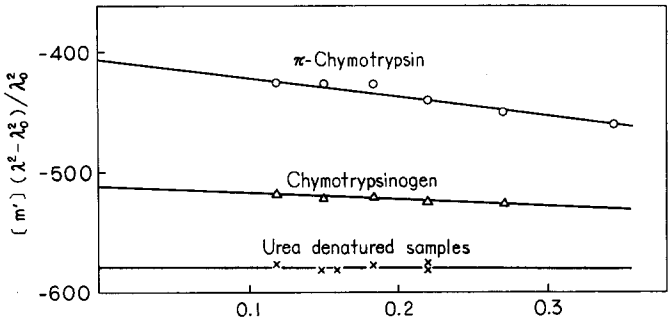


Fig. 1. Fit of equation (1) to the results of measurement of rotation. Medium for chymotrypsinogen and $\pi$-chymotrypsin: 0.05 $M$ phosphate buffer (pH 7.7) containing 0.1 $M$ $\beta$-phenylpropionate. For urea-denatured samples of both preparations: 8 $M$ urea, pH 7.7.

in Fig. 1. The results obtained are shown in Table I, from which it can be seen that the activation of the zymogen is accompanied by a 2-fold increase of the helical content. Since the molecular weights of chymotrypsinogen and $\pi$-chymotrypsin are about 24,000, it is concluded that a helical fraction of 5 to 6 turns is formed by the activation. It is noteworthy that such a small fraction is essential for the manifestation of the activity of this enzyme.

TABLE I

HELICAL CONTENT OF NATIVE AND UREA-DENATURED CHYMOTRYPSINOGEN AND $\pi$-CHYMOTRYPSIN

| | $-[\alpha]D$ | $-f^H\, b_0{}^H/600$ | $f^H\, a_0{}^H/680$ |
|---|---|---|---|
| Chymotrypsinogen | 87.4 | 0.12 | 0.11 |
| $\pi$-chymotrypsin | 74.3 | 0.23 | 0.26 |
| Urea-denatured chymotrypsinogen | 105.0 | 0.00 | 0.00 |
| Urea-denatured $\pi$-chymotrypsin | 104.3 | 0.00 | 0.00 |

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