THE ULTRAVIOLET ABSORPTION SPECTRUM OF PEPSIN

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I

In connection with measurements of the inactivation (destruction) of dissolved crystalline pepsin by ultraviolet light, determinations were made of the absorption spectrum of preparations of pure crystalline pepsin. From these data, the molecular extinction coefficient curve may be deduced. The pepsin used was prepared by Dr. John H. Northrop; through his kindness sufficient amounts of several preparations were made available (*cf.* Northrop, 1929–30*a*, *b*).

The crystalline pepsin in known dilutions in a suitable buffer solvent was put into 2 cm. glass photometer tubes or into adjustable micro Baly tubes with quartz end-plates. Corresponding control tubes were filled with the same dilution of the solvent solution. The tubes were placed in the paths of twin beams of light in a quartz sector photometer. The light source was a tungsten steel spark, 3 mm. gap, heated with a gas flame, and operated at 6600 volts from a transformer taking 17 amperes at 110 volts on the primary winding.

The transmission spectra were recorded on panchromatic photographic plates, using a large quartz spectrograph (Judd-Lewis, 1919, 1922; *cf.* Gates, 1930–31). The ratio of intensities incident on the two tubes was varied by means of the photometer sector vanes, over a wide range of intensities. When the length of liquid traversed by the light is kept constant the extinction coefficient is calculated from Beer's law. The logarithm of the ratio (I/I_o) is equal to log 1 − log D; log D is obtained from the scale readings on the vanes of the

* This paper is one of several in which results of work completed by Dr. Frederick L. Gates before his death, June 17, 1933, are reported. The manuscripts have been prepared by Professor W. J. Crozier and Dr. R. H. Oster.

photometer. The molecular extinction coefficients were calculated for the points at which bands of equal density appeared on the spectrograms and then plotted against the corresponding wave-lengths to give the absorption curve.

II

Tests were made on a preparation of carefully purified pepsin received from Dr. Northrop in 75 per cent glycerine, containing 1.80 mg. of protein nitrogen per ml., and on samples dissolved in M/100 HCl to make up dilutions of 1/10, 1/50, 1/100. By the quinhydrone electrode the 1 in 50 dilution showed a pH of 2.54; for the other dilutions this was not determined. For these solutions corresponding dilutions of M/100 HCl containing glycerine in the same proportion were used in the control tubes. From the spectrograms obtained with these samples between wave-lengths 2166 and 3130 Å.u., and from the calculated values of the molecular extinction coefficients, the absorption curve for pure pepsin was plotted (Fig. 1). On the basis of a molecular weight of 36,000 (Northrop, 1929–30*b*, p. 771), and the percentage of protein nitrogen of 15.15 (Northrop, 1929–30*a*, p. 747), the molecular extinction coefficient is calculated from the expression

$$\epsilon = \frac{\text{Log } D}{d \cdot M \cdot \text{dilution}}$$

where d is the thickness of solution and M is the molarity, in this case 0.00033 M; $M \times$ dilution is of course identical with C in the formula as usually written.

III

Slightly different values were obtained at high dilutions (1/125, 1/400) in some cases and with less highly purified preparations, but these irregularities were probably due either to pH differences or to uncertainty in regard to the concentration of the enzyme. In all preparations the location of the maxima and minima on the absorption curve coincided.

Other tests showed that even slight differences in the pH had a decided effect on the absorption of ultraviolet energy as indicated by the rate of inactivation of the enzyme. Northrop (1933–34) has shown

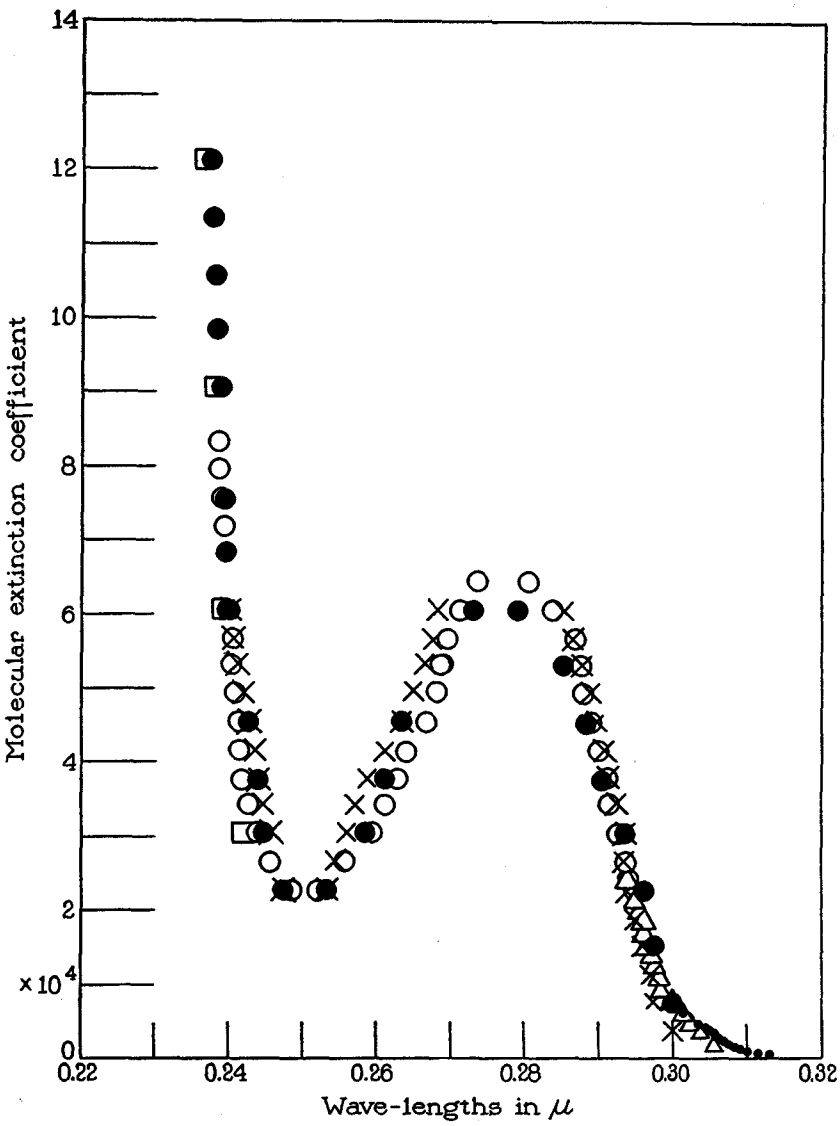

FIG. 1. The absorption curve of carefully purified crystalline pepsin (1.80 mg. of protein nitrogen per ml.) in 75 per cent glycerine dissolved in M/100 HCl to give the following dilutions: Clear circles and solid circles represent points obtained with two solutions of pepsin diluted 1 in 50, pH 2.54, and tested in 2 cm. photometer tubes; small dots, undiluted pepsin solution tested in 1 cm. tubes; crosses, diluted to 1 in 50, pH 2.54, and tested in 4 cm. tubes; triangles, diluted to 1 in 10 and tested in 2 cm. tubes; squares, diluted to 1 in 100 and tested in 2 cm. tubes. The range of intensities on the photometer sector scales was spaced at intervals of $\log_{10} D = 0.1$, between 0.1 and 1.6, to cover the complete range plotted.

that the rate of inactivation depends on the pH of the solution, and this has also been indicated by Collier and Wasteneys (1932) and by Pincussen and Uehara (1928).

IV

As shown in Fig. 1 the absorption approaches a maximum in the region below 2400 Å.u.; a lower peak of absorption occurs at 2750–2800 Å.u.; minimum absorption is near 2500 Å.u. and in the region of wave-lengths longer than 3000 Å.u.

Kubowitz and Haas (1933) determined the absorption curve of urease in the ultraviolet, and measured the decrease in activity of a solution of jack-bean urease (Sumner, 1926) when irradiated with ultraviolet light of measured intensities at wave-lengths between 196 mμ and 366 mμ. They computed from these data the relative absorption spectrum of urease. Since the molecular weight of urease was not known, close comparison of their curve with that found for pepsin is not possible. However, the locations of maximum and minimum of absorption seem to agree rather closely. This is in accordance with the similarities implied in the protein character of the two enzymes, and in the agreement of their crystalline forms, as pointed out by Northrop (1929–30*a*).

The slight but definite changes in the slope of the curves (humps) at 2560–2650 Å.u., at 2930 Å.u., and at 3000 Å.u. may be significant as indicating a difference between the absorption spectrum of pepsin and that of such a substance as tyrosine. These humps may also indicate certain fine characteristics in the absorption of ultraviolet energy associated with enzyme activity, of the type found by Lavin, Northrop, and Taylor (1933) in the absorption spectrum of pepsin at low temperatures.

SUMMARY

The ultraviolet absorption spectrum of Northrop's pure crystalline pepsin has been determined. The curve of calculated molecular extinction coefficients is given. There is noted a general resemblance of the absorption curve for pepsin to that for urease and tyrosine; the absorption band is maximum at 2750–2800 Å.u., minimum near 2500. A slight hump on either side of the peak of the extinction curve may be significant.

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