ABSORPTION SPECTRUM OF THE PEPTIDE BOND

II. INFLUENCE OF CHAIN LENGTH

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In an earlier report (1) it was shown that a band characteristic of the peptide bond appeared in the ultraviolet and that values of the molar absorption coefficient ($\epsilon_m$) at 205 m$\mu$ could be used to characterize this band. It was shown, for a series of proteins, that the contribution of each peptide bond ($\epsilon_p$) to the molar absorption coefficient is fairly constant, lying between 2500 and 2800. The large value of $\epsilon_p$ for glycylglycine (3300) was attributed to an interaction of the charges on the dipole ion which was assumed to affect the peptide bond, causing an increase in $\epsilon_p$. As a confirmation of this it was pointed out that, when the charges were separated further, as in triglycine, the value of $\epsilon_p$ dropped to about 2500, and, where no dipole existed, as in acetylglycine, the value of $\epsilon_p$ dropped even further. It was thought desirable to reexamine the effect of chain length and it has been found that, contrary to the earlier conclusion, the value of $\epsilon_p$ does vary with the number of amino acid units in the peptide chain.

EXPERIMENTAL

Materials

The poly-L-lysine and poly-DL-ornithine samples were supplied as the hydrochlorides by Dr. M. A. Stahmann and Dr. E. Katchalski. The samples were dried over P$_2$O$_5$ in a vacuum desiccator and used without further purification. All other chemicals were c.p. or A. C. S. grade.

Methods—The polyamino acids were dissolved in solutions of approximately 1, 2, 4, 6, and 8 pH. The first two were obtained by use of 0.1 N and 0.01 N H$_2$SO$_4$. The last three were 0.005 m phosphate solutions of the desired pH. The exact pH was determined with a Beckman pH meter, model No. H2, with a glass electrode.

RESULTS AND DISCUSSION

Absorption curves for polylysine ($n = 6, 14, 33, 58,$ and 83)$^2$ and polyornithine ($n = 35$) were obtained by averaging the values of $\epsilon_m$ for the

1 The author is indebted to Dr. Katchalski of Harvard University for the sample of polylysine, $n = 33$ (2), and polyornithine, $n = 35$ (2), and to Dr. Stahmann of the University of Wisconsin for polylysine samples with $n = 6, 14, 58,$ and 83 (3).

2 $n$ represents the average number of amino acid residues per mole.
whole pH range studied. The curve for polylysine, \( n = 6 \), is taken as the average at pH 4, 6, and 8 (Fig. 1). The values for \( \epsilon_m \) for hexalysine at pH 1 and 2 were lower and the significance of this will be discussed below. The shapes of all the curves are the same. The corrected values of \( \epsilon_p \) at 205 m\( \mu \) for each of the compounds are given in Table I. With the exception of polylysine, \( n = 33 \), all the values of \( \epsilon_p \) are in the range found for proteins (between 2500 and 2800). In view of the "normal" value of \( \epsilon_p \) for polyornithine, \( n = 35 \), it is believed that the high value for polylysine,
$n = 33$, is not significant and is due primarily to a high absorbing impurity. These results would have seemed to confirm the earlier conclusion that $\epsilon_p$ is independent of the number of residues, except that it is necessary to account for the pH effect on $\epsilon_m$ for lower peptides.

It was stated above that $\epsilon_m$ for hexalysine increases in going from a solution of low pH to about neutrality. A similar observation was made earlier for glycyglycine (1) and triglycine. Complete $\epsilon_m$ versus pH for hexalysine was obtained and the results are given in the curves in Fig. 2. The calculated value of pK for triglycine is 3.40 and for hexalysine 2.64. These are both in the range of values for peptide pK$_a$ (4). The reported value of pK$_a$ for triglycine is 3.26 and for lysyllysine 1.95. It would therefore appear that the difference of $\epsilon_m$ with changing pH is due to the ionization of the carboxylic acid group. The molar absorption coefficient would appear to be the sum of $(n - 1) \times \epsilon_p$ plus $n \times$ (side chain absorption) plus the contribution of the carboxylic acid group. The difference between $\epsilon_m$ at pH about 1.0 ($\Delta \epsilon_m$) gives the contribution due to the conversion of COOH $\rightarrow$ COO$^-$. For triglycine $\Delta \epsilon_m \approx 2140$ and for hexalysine about 2000. Since $\Delta \epsilon_m$ remains somewhat constant, at higher values of $n$ there should be, and is, a point at which there is no change of $\epsilon_m$ between a low pH and neutrality and this corresponds with the data for polylysine ($n = 14$ to 83) and polyornithine ($n = 35$). According to this reasoning, it would seem best to calculate $\epsilon_p$ for peptides from $\epsilon_m$ at a low pH (about 1.0). The results calculated show that the contribution of the peptide band to $\epsilon_m$ for triglycine is 2960, and for hexalysine 10,600. Therefore, for triglycine $\epsilon_p$ is 2960/2 or 1480 and for hexalysine 10600/5 or 2120. It has already been pointed out (1) that acetylglycine has $\epsilon_m$ of 1100 and that glycyglycine in 0.1 N HCl is 1490. Corrected for side chain absorption, $\epsilon_p$ for acetylglycine is 1000 and for glycyglycine 1390. From these data it would appear that $\epsilon_p$ increases with the number of amino acid residues to a constant value lying between 2500 and 2800. A word of caution should be mentioned; namely, that the large value of $\Delta \epsilon_m$ is only for a terminal COOH in a peptide chain. It has been shown that amino acids and simple carboxylic acids do not increase $\epsilon_m$ more than about 100 at the COO$^-$ stage (pH $\sim$6) compared to the unionized form (pH $\sim$1) (5).

**SUMMARY**

1. The absorption spectra of polylysine, $n = 6, 14, 33, 48,$ and 53, and polyornithine, $n = 35$, are reported.

The values are calculated from the data with the expression pH = pK $+$ log $\left(\frac{\epsilon_m - \epsilon_H}{\epsilon_A - \epsilon_m}\right)$, where $\epsilon_m$, $\epsilon_H$, and $\epsilon_A$ are molar absorption coefficients; experimental, the constant values at low pH and high pH respectively. The results of this calculation agree with the extrapolated values within a 0.1 pH unit.

1 This has been discussed (1).
2. The $\epsilon_m$ versus pH curves for hexalysine and triglycine are reported at between about pH 1 and 7. The change in $\epsilon_m$ is correlated with the ionization of the carboxylic acid group.

3. It is concluded that the contribution of each peptide group, $\epsilon_p$, is smaller for the smaller peptides and rises to a constant value.

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BIBLIOGRAPHY

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