TABLE I

APPARENT SECOND ORDER RATE CONSTANTS AS A FUNCTION OF EXTENT OF REACTION

In units of M^{-1} sec.$^{-1}$ × 10^{-7}, under conditions given in Text.

	ε-N-DNP-L-Lysine			N-DNP-ε-Aminocaproate		
Initial ratio [Hp]/[Ab sites] →	0.5	2.0	8.4	0.5	2.0	8.1
Fraction of total Ab sites reacted						
0.1	9.0	..	..	13.2	..	..
.2	7.9	..	..	11.1	..	..
.4	5.8	6.8	..	9.9	9.0	..
.6	..	6.2	3.9	..	7.7	5.9
.8	..	2.5	2.0	..	5.1	2.1
.9	..	..	1.4	..	..	1.5

in net electric charge of pure anti-DNP Ab[3] is not primarily responsible for the apparent kinetic heterogeneity).

The maximum value of k_1 for both haptens appears to be about 1×10^8 M^{-1} sec.$^{-1}$, which makes this one of the most rapid bimolecular reactions in homogeneous solution known to biochemistry. It is interesting that the same k_1 value was estimated[1] from stopped-flow experiments with anti-DNP Ab and an entirely different DNP-hapten, 2-(2,4-dinitrophenylazo)-1-naphthol-3,6-disulfonic acid, by an independent spectrophotometric technique. One would predict[11,12] for the present system that diffusion would limit the rate constant to a value of about 10^9 M^{-1} sec.$^{-1}$. Such a large value for k_1 suggests that no substantial conformational rearrangement (requiring an appreciable free energy of activation) occurs within the Ab molecule, or active site, upon specific binding of the hapten.

This research was aided by grants RG-4725 (J.M.S.) and E-4246 (S.J.S.) from the National Institutes of Health, U. S. Public Health Service.

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RECEIVED AUGUST 6, 1962

CONFORMATIONAL ASPECTS OF SYNTHETIC POLYPEPTIDES. VI. HYPOCHROMIC SPECTRAL STUDIES OF OLIGO-γ-METHYL-L-GLUTAMATE PEPTIDES[1]

Sir:

Absorption spectra of high molecular weight polypeptides and proteins in the far ultraviolet region have been employed for the study of their conformations in solution.[2-6] Peptides in the helical conformation have been shown to exhibit a marked decrease in intensity of the absorption band (molar extinction coefficient approximately 4,000) in the 190 mμ region compared with the same peptide in random coil conformation (molar extinction coefficient approximately 7,500). Helical structures also exhibit a new absorption band or shoulder in the 205 mμ region. Polylysine, polyglutamic acid and polyalanine are examples of synthetic polypeptides which have been studied by this technique.

We have applied the study of far ultraviolet absorption spectra to oligomeric peptides derived from γ-methyl-L-glutamate where the amino end is blocked by a benzyloxycarbonyl group.[1,7] All evidence which we have developed to date on the critical size necessary for helix formation has depended on optical activity measurements. The application of hypochromic studies from absorption spectra described in this paper provides the first data for the independent verification of the onset of helical conformations in the critical range of peptide chain length.

The solvent for our investigations was 2,2,2-trifluoroethanol which has low absorption in the 190 mμ region. A Perkin Elmer model 350 spectrophotometer was used in this work. It was kept air free by continuous flushing with dry prepurified nitrogen. This enabled us to work in the region down to 189 mμ with a slit width of less than 0.6 mm. and to 187 mμ with a slit width of less than 1.5 mm. Matched quartz cells (0.1 mm. width) were used. The concentrations of the peptides studied were in the range of 10^{-2} to 10^{-3} mole/liter.

The molar extinction coefficients (ϵ') are shown in Fig. 1 as a function of wave length for various oligopeptides and for a high polymer of γ-methyl-L-glutamate. These extinction coefficients (ϵ') have been corrected for the absorption of the benzyl group of the benzyloxycarbonyl blocking group in the oligopeptides.[8]

$$\epsilon'_{\text{peptide}} = \epsilon_{\text{observed}} - f_{C_6H_5CH_2} \times \epsilon_{C_6H_5CH_3}$$

where ϵ' is the molar extinction coefficient per residue corrected for benzyl group absorption. $\epsilon_{\text{observed}}$ is the observed molar extinction coefficient. $f_{C_6H_5CH_2}$ is the weight fraction of the benzyl group in the molecule. $\epsilon_{C_6H_5CH_3}$ is the molar extinction coefficient of toluene in this region.

With the peptides studied the hypochromicity of the absorption band measured at 189–190 mμ commences at the nonamer, increasing in magnitude for the higher oligopeptides and the polymer. The appearance of the shoulder at the 205 mμ region also begins at the nonamer, increasing in intensity with chain length.

Assuming that the high polymer exists entirely in the helical state and the pentamer and heptamer possess no helical conformation in this solvent, we have calculated the helical content for any of the oligomers from the extinction coefficients at

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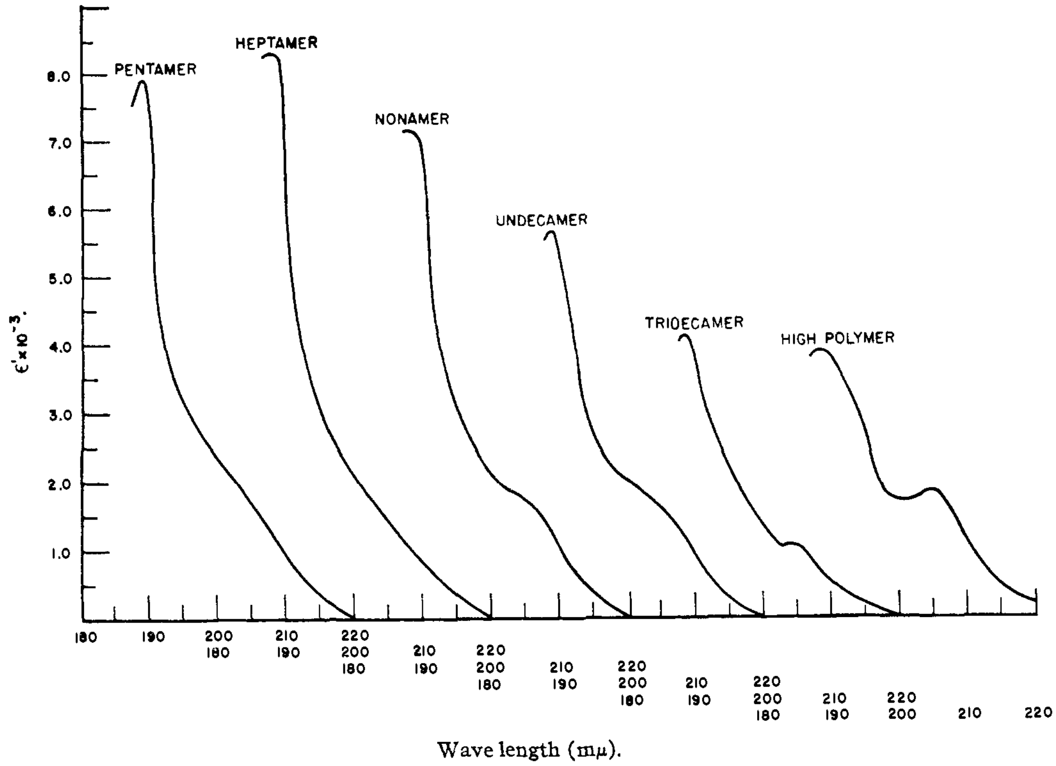

Fig. 1.—Molar extinction coefficients per residue of the oligomeric peptides and high polymer derived from γ-methyl-L-glutamate as a function of wave length in 2,2,2-trifluoroethanol at 25.0°.

189 mμ using the relationship[2]

$$(\epsilon_c' - \epsilon')/(\epsilon_c' - \epsilon_h') \times 100 = \%\ \text{helicity} \quad (1)$$

where ϵ' is the molar extinction coefficient per residue of the peptide, ϵ_c' is the molar extinction coefficient per residue of the random coil (based on the hepta and pentapeptides). ϵ_h' is the molar extinction coefficient per residue for the helical peptide (based on the polymer).

The data in Table I summarize the various absorption coefficients and the % helicity for the compounds using Equation 1.

TABLE I

ABSORPTION COEFFICIENTS, PERCENT HELICITY AND ROTATORY DISPERSION CONSTANTS FOR THE PEPTIDE SERIES IN TRIFLUOROETHANOL AT 25°

Compound	Molar extinction coefficients at 189 mμ ϵ(observed) $\times 10^{-3}$	ϵ'(corrected) $\times 10^{-3}$	% Helicity $(\epsilon_c' - \epsilon')/\epsilon_c' - \epsilon_h' \times 100$	Rotatory dispersion constants b_0
Dipeptide	17.3	7.1	(0)[a]	...
Tripeptide	..	..	...	+72
Tetrapeptide	..	..	...	+50
Pentapeptide	13.3	7.9	(0)[a]	0
Hexapeptide	..	..	...	0
Heptapeptide	12.3	8.2	(0)[a]	−42
Nonapeptide	10.4	7.1	26	−210
Undecapeptide	8.4	5.6	60	−277
Tridecapeptide	6.5	4.1	95	−337
Polymer	3.9	3.9	100	−816

[a] The helical content is zero or close to it.

The work of Tinoco, Halpern and Simpson[2] indicates that for very short chains the hypochromism calculated for the complete helix is somewhat lower than that calculated for the completely helical high polymers. Since this variation of hypochromism does not have much of an effect on our calculations of percentage helicity, we assume the hypochromism of the complete helix to be independent of the number of residues in the peptide chain.

These findings which we are reporting are in accordance with our studies using optical rotatory properties of the oligopeptides in other helical solvents.[1,7] Optical rotatory studies in 2,2,2-trifluoroethanol also were carried out. It is evident (Table I) that there is excellent agreement between the rotatory dispersion data and the results obtained from the ultraviolet spectra. The appearance of helical structures in trifluoroethanol at 25° commences at or about the monamer as can be seen by the abrupt change in b_0 and the extinction coefficients (Table I).

Acknowledgment.—We wish to thank Dr. I. Tinoco for his extremely helpful suggestions. We also wish to acknowledge gratefully the support given for this research by a grant from the National Institutes of Health RG 8974.

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RECEIVED JUNE 27, 1962

A CONVENIENT SYNTHESIS OF N-ALKYLHYDROXYLAMINES

Sir:

We wish to report a new synthesis of N-monosubstituted hydroxylamines by reduction of the