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Ultraviolet Study of the Solvent Induced Coil to Helix Transition of Poly(α-L-Glutamic acid)

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Summary

The coil to helix transition of polypeptides can be induced by lowering the pH in pure aqueous solutions, or by adding an organic solvent to an aqueous solution of the polypeptide(starting pH higher than 7.0 for poly-α-L-glutamic acid)(MORCELLET and LOUCHEUX 1978a). It has been shown since many years that the pH induced transition of poly(α-L-glutamic acid) (PGA) may be followed by U.V. spectroscopy since the $\pi \rightarrow \pi^*$ absorption band of the peptide chromophore near 190 nm exhibits strong hypochromism when the polypeptide is under the helical conformation (IMAHORI and TANAKA 1959). Similar results have been also obtained about an other extensively studied polypeptide, poly-L-lysine (ROSENHECK and DOTY 1961, CHOU and SCHERAGA 1971). The present paper shows that the solvent induced transition of PGA may also be monitored by U.V. spectroscopy.

Introduction

Since the early works of GOLDFARB and SAIDEL (1951) on proteins, of IMAHORI and TANAKA (1959) and HOLTZWARTH and DOTY (1965) on polypeptides, it is now well known that the denaturation of proteins or the conformational transition of polypeptides induces changes in the absorption properties of the macromolecule especially of the peptidic chromophore. IMAHORI and TANAKA (1959) have shown that the two thirds of the 40 % hypochromism of the helical conformation of PGA compared to the random coil conformation (ε_{190} = 4000 $l.cm^{-1}mole^{-1}$ for the helix and ε_{190} = 7000 $l.cm^{-1}mole^{-1}$ for the random coil) is due to changes in the absorption properties of the peptide chromophore. The remaining third originates in the difference between the absorption of the carboxylic group (ε_{190}= 27 $l.cm^{-1}mole^{-1}$) and of the carboxylate group (ε_{190} = 1400 $l.cm^{-1}mole^{-1}$), i.e. is the absorption changes during the ionization of the polypeptide, independently of the conformational changes. On the other hand, it is well known that the conformational transition of PGA may also be induced

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by the addition of an organic solvent, such as dioxane, to an aqueous solution of the ionized polymer(M.MORCELLET and C. LOUCHEUX 1978 a).

This paper presents the results obtained concerning the U.V. absorption of PGA and its sodium salt in water dioxane mixtures. In order to distinguish between the contributions of the two chromophores of PGA (i.e. the peptide group and the carboxyl group) and the contribution of conformational changes some measurements were also carried out on model molecules : acetic acid, N acetyl glycine (CH_3-Co-NH-CH_2-COOH) and their sodium salts.

Experimental

The PGA sample was prepared by debenzylation of poly(γ benzyl-L-glutamate) according to IDELSON and BLOUT (1958). Its molecular weight was $\bar{M}_w$ = 33 000 according to the viscosity method of WADA (1960). The sodium salt of PGA (PGANa) was prepared by neutralization with sodium hydroxide, followed by exhaustive dialysis against pure water. N-acetyl glycine (NAG) was prepared by reaction of acetyl chloride with glycine (MERCK) and then recristallised from hot water. N-acetyl glycine sodium salt (NAGNa) was obtained by neutralization with sodium hydroxide. All other chemicals were commercial products (Acetic acid, sodium acetate and dioxane, spectroscopy grade, MERCK).

U.V. spectra were recorded with a Cary 118 U V spectrophotometer, flushed with dry nitrogen. 0.01 cm cells were used to limit the absorption of dioxane. Special care was taken to remove oxygen and carbon dioxide from the solutions. In these conditions, U.V. spectra were recorded down to 180 nm with pure aqueous solutions and down to 200 nm with water-dioxane solutions.

Results and discussion

Figure 1 shows a comparison between U.V. spectra of PGA, PGANa, acetic acid and sodium acetate. The peptide chromophore presents two electronic transitions in this domain : the $n \rightarrow \pi^*$ transition at about 220 nm and the $\pi \rightarrow \pi^*$ transition at about 190 nm.(HAM and PLATT 1958, HUNT and SIMPSON 1953, PETERSON and SIMPSON 1956, GRATZER 1967, KLYNE and SCOPES 1973). The carboxyl chromophore has a $n \rightarrow \pi^*$ transition in the 190-210 nm spectral range (at 205 nm for CH_3COOH, see Fig. 1) (JAFFE and ORCHIN 1962). U.V. spectra in Fig. 1 cleary show that the difference between the helical and the coil conformation of PGA cannot be accounted for by the only ionization of the carboxyl group : For PGA and PGANa, $\Delta\varepsilon$ at 190 nm is 3000 $mole^{-1}.l.cm^{-1}$; for acetic acid and sodium acetate, $\Delta\varepsilon$ at 190 is about 1300 $mole^{-1}.l.cm^{-1}$. Thus most of the difference is due

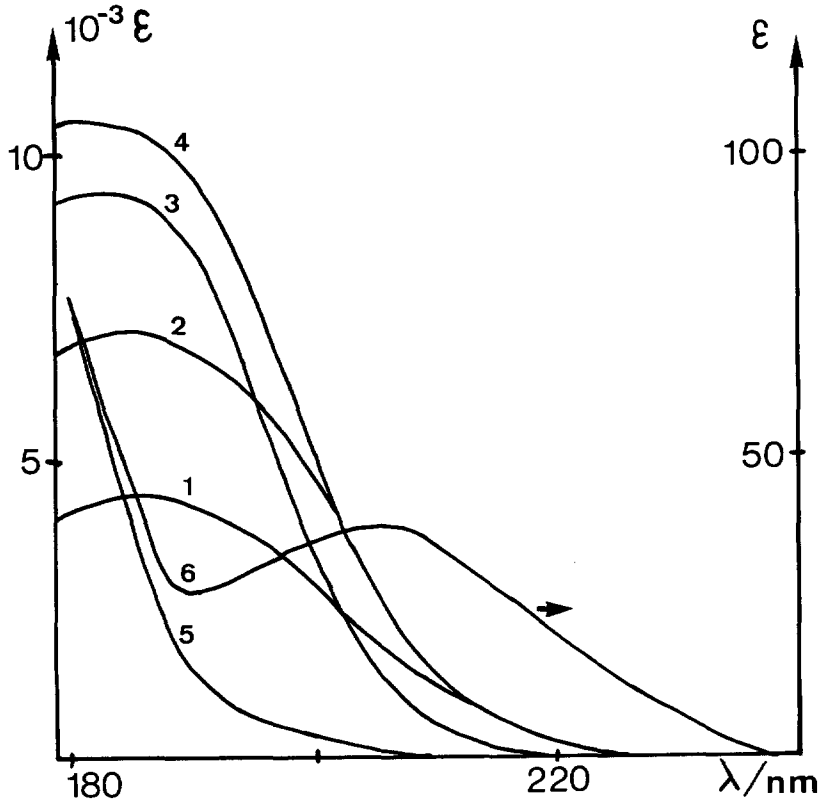

Figure 1. U.V. spectra of PGA (1), PGANa (2), NAG(3), NAGNa (4), sodium acetate (5) (left scale), and acetic acid (6) (right scale)

to the unfolding of the helical conformation, or, more precisely, to the hypochromism of the peptide chromophore when it is arranged in a regular conformation such as the α helical conformation. Further confirmation is given by the U.V. spectra of NAG and NAGNa : at 190 nm $\Delta\varepsilon$ is equal to 1250 $mole^{-1}.l.cm^{-1}$, i.e. the same difference as for acetic. Thus the absorption of the peptide chromophore is unaffected by the ionization of the molecule, which cannot take any regular conformation. It can pointed out that in these conditions the protonation of NH groups cannot occur.

Figure 2 and Table 1 give the variation of the molar extinction coefficient ε as a function of the volume fraction of dioxane for PGA, PGANa, NAG, NAGNa, CH_3COOH and CH_3COONa. For PGANa ε decreases between 0 and 40 % of dioxane then increases until 80 %. This is in good agreement with the dioxane induced coil to helix transition of PGANa which occurs near 40 % of dioxane added (MORCELLET and LOUCHEUX 1975). Thus, this transition is accompanied by changes in the U.V.absorption of the polymer.

TABLE 1

Molar absorption coefficient at 205 nm as a function of the volume fraction of dioxane for CH_3COOH, CH_3COONa, NAG and PGA (ε in $l.mole^{-1}.cm^{-1}$)

Vol.% of dioxane	CH_3COOH	CH_3COONa	NAG	PGA
0	37	180	1420	1900
20	36	190	1350	1850
40	37	210	1240	1910
60	37	210	1310	1900
80	36	200	1360	1880

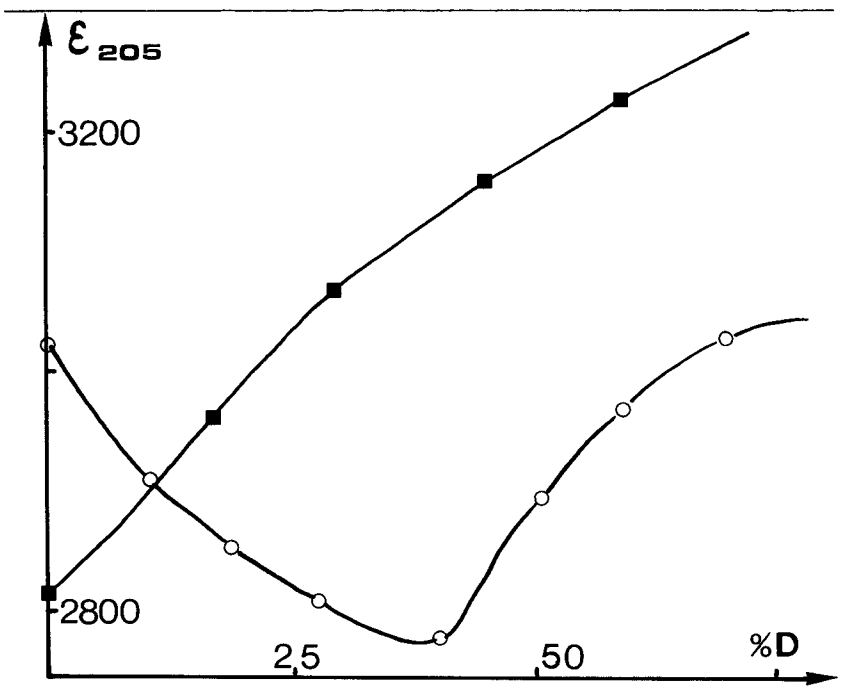

Figure 2a. Variation of ε_{205} for PGANa (○) and NAGNa (■) in water dioxane mixture

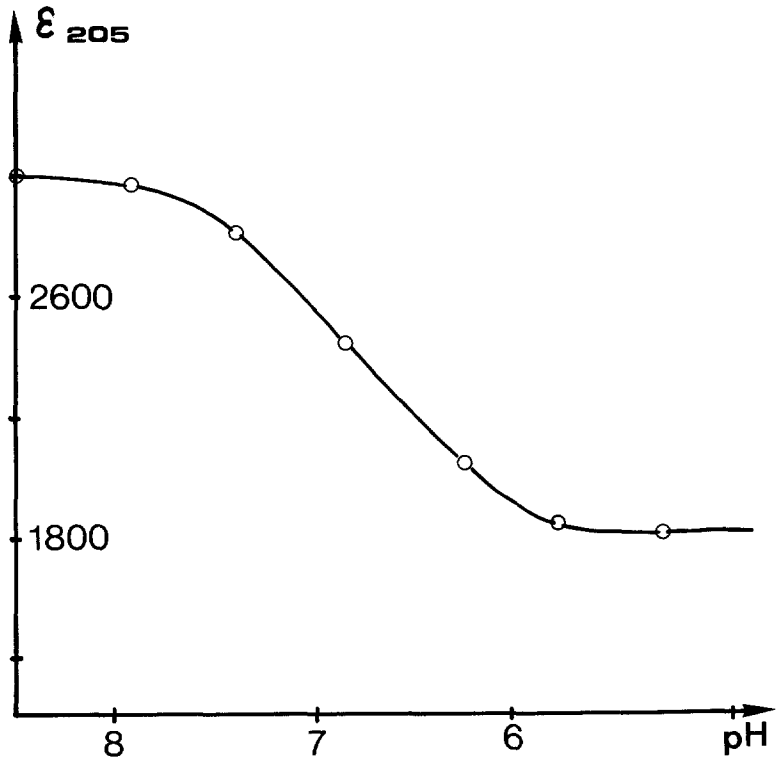

Figure 2b. Variation of ε_{205} for PGANa versus pH (from IMAHORI and TANAKA)

Figure 2 also shows that, for NAGNa for which no conformational transition occurs, ε_{205} increases regularly between 0 and 80 % of dioxane. Likewise no important change is observed in the values of ε for CH_3COOH, CH_3COONa, NAG and PGA (Table 1). Thus dioxane has no direct influence on the absorption properties of the carboxyl and carboxylate group as well as the peptide chromophore when it is included in an unionized molecule. On the contrary, ε depends on the composition of the water-dioxane mixture for ionized molecules containing the peptide chromophore as shown by NAGNa and PGA (Figure 2). On the other hand, it has been shown in previous works that many solution properties of

PGANa in water-dioxane mixture depend on the preferential or absolute solvation of the polymer by dioxane. (MORCELLET and LOUCHEUX 1975, MORCELLET and LOUCHEUX 1978b). Especially, it has been shown that PGANa is strongly solvated by dioxane. Among other consequence, it lowers the local dielectric constant around the polymer and this results in an increase of the molar extinction coefficient of the peptide group (JAFFE and ORCHIN 1962). This explains the increase of ε_{205} for NAGNa shown in Fig. 2. It must be noted that ε_{205} for PGANa in 80 % dioxane is nearly equal to ε_{205} in pure water ($\sim$ 3050 $l.mole^{-1}.cm^{-1}$), whereas for the pH induced transition, it decreases from 3000 to 1800 $l.mole^{-1}$ cm^{-1} when going from coil to helix. This is the result of the increase of ε due to the lowering of the dielectric constant which balance the lowering of ε due to the conformational change.

The decrease in ε between 0 and 40 % of dioxane for PGANa is not observed for NAGNa. It could be the result of the important changes in the molecular dimensions of PGANa which occur in this domain (MORCELLET and LOUCHEUX 1976).

To conclude it can be asserted, by comparison with the results obtained for the model molecules NAG and NAGNa, that the variations of ε observed for PGANa are due to the solvent induced conformational transition undergone by the polymer, as it was previously shown for the pH induced transition (IMAHORI and TANAKA 1959). Thus the measurement of hypochromism is a convenient tool to investigate solvent-induced coil to helix transitions too.

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