Ultraviolet Rotatory Properties of Synthetic Polypeptides in Solution. II. The Behavior of Poly(1-benzyl-L-histidine) in Trifluoroethanol in the Presence of Different Acids[1]

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ABSTRACT: The circular dichroism and nmr properties of poly(1-benzyl-L-histidine) (PBLH) and of random copolymers of 1-benzyl-L-histidine with N^{ϵ}-carbobenzoxy-L-lysine in trifluoroethanol solutions have been investigated in the presence of $HClO_4$, H_2SO_4, HCl, CF_3COOH, and hexafluoroacetone sesquihydrate. It was found that, only in the presence of a stoichiometric amount of $HClO_4$ with respect to the histidine residues, pure PBLH assumes the ordered conformation called PBLH II. On the contrary, equivalent amounts of HCl, H_2SO_4, and CF_3COOH cause the random coil conformation to be formed. The fluoroacetone compound, which is a weak acid in the solvent medium we used, has no effect on the polymer conformation, at least until [acid]/[His] ratios are equal to 2. The results are interpreted in terms of specific interactions of perchlorate ions with the charged side-chain groups of the polymer.

In a previous investigation[1] we have studied the conformational properties of poly(1-benzyl-L-histidine) (PBLH) in various solvent media. On the basis of circular dichroism (CD) data obtained for the pure homopolymer and for random copolymers of 1-benzyl-L-histidine with N^{ϵ}-carbobenzoxy-L-lysine, the conclusion was drawn that PBLH in trifluoroethanol (TFE) assumes the right-handed, α-helical conformation. The most interesting result was that the pure polymer assumes a new, unknown conformation, which has been named PBLH II, when the stoichiometric amount of $HClO_4$ (with respect to the histidine residues) is added to the solution in TFE. The variation of the CD properties of the polymer as a function of the amount of $HClO_4$ unambiguously showed that the conformation of PBLH II must be an ordered one. After the above-mentioned work two main problems remained open. First, it was not clear if protonation of the 1-benzylimidazole side chains is a necessary and sufficient condition for the conformational stability of PBLH II in TFE. Second, it was not possible to draw conclusions on the exact type of conformation corresponding to PBLH II.

In the present paper we report the results of extensive investigations on the first problem, namely, on the effect of different acids on the conformation of PBLH in TFE. We studied the effects of H_2SO_4, HCl, CF_3COOH (TFA), and of hexafluoroacetone sesquihydrate (HFAS) on the conformational properties of PBLH and of random copolymers of 1-benzyl-L-histidine with N^{ϵ}-carbobenzoxy-L-lysine, in connection with the extent of protonation of the polymer and copolymer side chains. CD and 60-MHz nmr techniques have been used. Information on the exact nature of the conformation of PBLH II will appear in a subsequent paper.

Experimental Section

Materials. TFE (Halocarbon Chemical Co., Hoboken, N. J.) and hexafluoroacetone sesquihydrate (HFAS) were of high purity and were used as received. Sulfuric acid, hydrochloric acid, and trifluoroacetic acid (TFA) were high purity products from Carlo Erba (Milan, Italy).

Poly(1-benzyl-L-histidine) (PBLH) and Random Copolymers of 1-Benzyl-L-histidine and N^{ϵ}-Carbobenzoxy-L-lysine. The preparation and the characterization of these materials have been described in detail in our previous paper.[1]

Measurements. The extent of protonation of the 1-benzylimidazole side chains of PBLH and of the copolymers in the presence of different acids has been followed by 60-MHz nmr measurements in TFE (downfield shift of the =CH– proton located between the two nitrogen atoms of the imidazole group).[1,2] Aqueous solutions (4 *N*) of the various acids were added to the polymer and copolymer solutions (of known concentrations) by using a Metrohm precision microburet, Model E 457. The polymer concentration was always around 8% (w/v). A Perkin-Elmer nmr spectrometer, Model R 12, has been used.

CD measurements were performed using either a Roussel-Jouan 185 Model II dichrograph or a Cary 60 spectropolarimeter equipped with a Model 6002 CD attachment. The readings were transformed into $\Delta\epsilon$ values using well-known equations.[3] The concentration of optically active material was always very close to 0.5 g/l. Either 0.5- or 1-mm fused quartz cells with Suprasil windows were used.

Results and Discussion

Protonation Studies on PBLH in TFE. Figures 1a–d show the effect of H_2SO_4, HCl, TFA, and HFAS on the nmr spectrum of PBLH in TFE. The protonation of the 1-benzylimidazole groups causes a large downfield shift of the =CH– proton (C-3) located between the two nitrogen atoms, and of the =CH– proton (C-2) located between the nitrogen and the carbon atoms. Protonation also affects to a minor extent the chemical shift of the phenyl protons (1). Figure 2 shows the relative chemical shift of the C-3 proton with respect to the phenyl protons as a function of the molar acid:histidine ratio. In the case of H_2SO_4 and HCl the downfield shift increases linearly with the amount of acid until an [acid]/[His] ratio near 0.7. The slope of the curve then diminishes gradually and becomes 0 (plateau region of Figure 2) at ratios higher than 1. In the case of TFA the chemical shift increases linearly with the amount of acid (and overlaps that observed with HCl and H_2SO_4) until [acid]/[His] ratios of *ca.* 0.2. The slope of the curve then diminishes continuously to a plateau region for [acid]/[His] ratios higher than 1. Finally, in the case of HFAS the chemical shift increases until an [HFAS]/[His] ratio of ~0.3 and then a plateau region is reached which is much lower on the ordinate scale with respect to the corresponding plateau regions of H_2SO_4, HCl, and TFA.

(1) For part I, see M. Terbojevich, M. Acampora, A. Cosani, E. Peggion, and E. Scoffone, *Macromolecules*, 3, 618 (1970).

(2) D. H. Meadows, J. L. Markley, J. S. Cohen, and O. J. Jardetzky, *Proc. Nat. Acad. Sci. U. S.*, **58**, 1307 (1967).

(3) L. Velluz, H. Legrand, and M. Grosjean, "Optical Circular Dichroism," Academic Press, New York, N. Y., 1965, p 62.

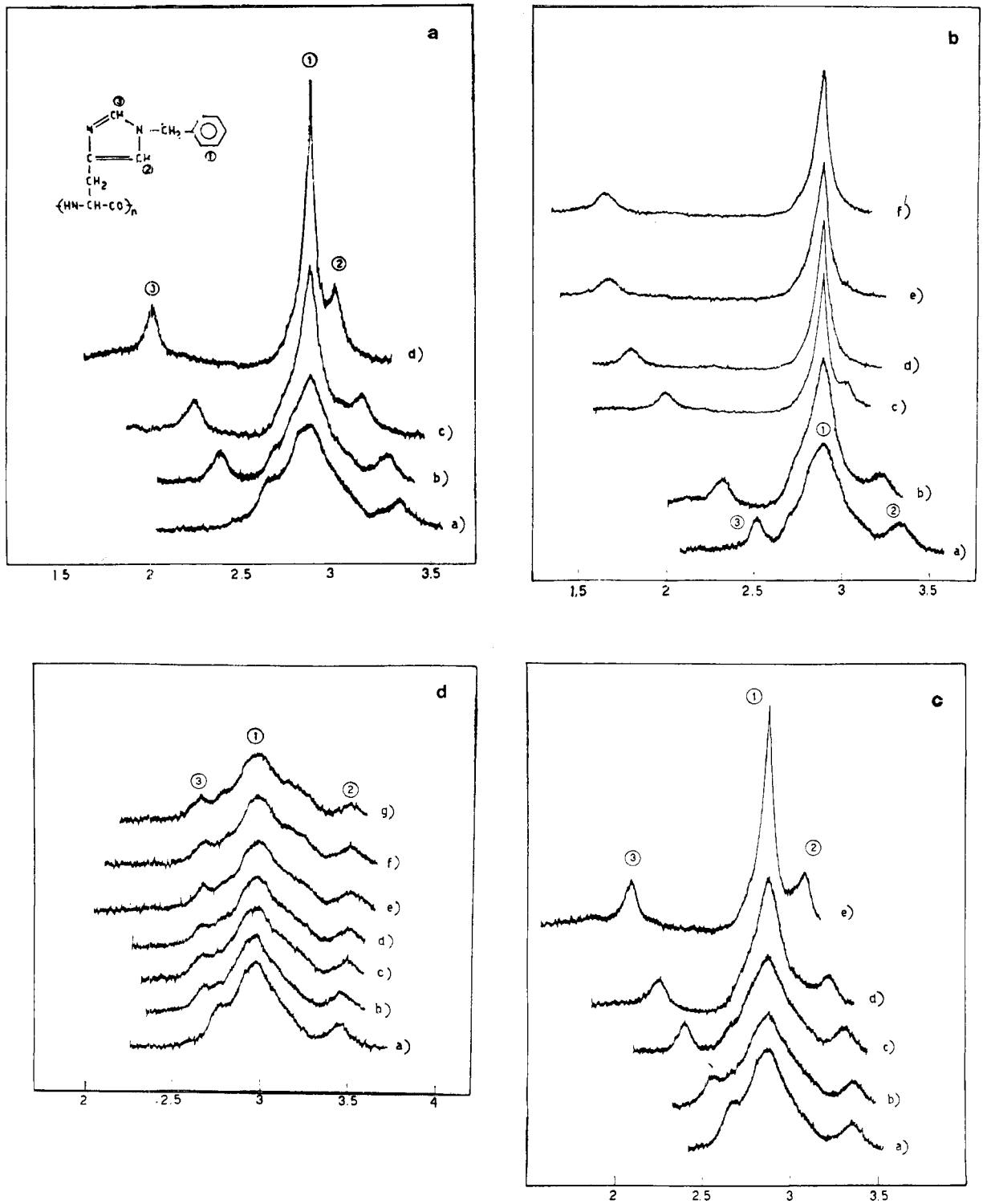

Figure 1. (a) Some typical 60-MHz nmr spectra of PBLH in TFE at various [HCl]/[His] equivalent ratios: a, 0 (no added acid); b, 0.230; c, 0.382; d, 0.535. In this and in the subsequent Figures 1b–d the spectra have been shifted in order to have exact overlapping of the peaks corresponding to the phenyl protons. (b) Some typical 60-MHz nmr spectra of PBLH in TFE at various [H_2SO_4]/[His] equivalent ratios: a, 0.154; b, 0.308; c, 0.538; d, 0.693; e, 0.847; f, 1.00. (c) Some typical 60-MHz nmr spectra of PBLH in TFE at various [TFA]/[His] equivalent ratios: a, 0 (no added acid); b, 0.077; c, 0.230; d, 0.390; e, 0.540. (d) Some typical 60-MHz nmr spectra of PBLH in TFE at various [HFAS]/[His] equivalent ratios: a, 0 (no added acid); b, 0.146; c, 0.292; d, 0.438; e, 0.584; f, 0.875; g, 1.17.

It is important to remark that the acids examined do not cause polymer precipitation from the 8% (w/v) solution used for nmr experiments, at least in the range of acid employed. This behavior is different from that observed with $HClO_4$, which causes PBLH to precipitate from the 8% solution when the [$HClO_4$]/[His] ratio is higher than 0.4.[1] The results of the nmr experiments indicate that $HClO_4$, H_2SO_4, HCl, and TFA have slightly different protonating capacity toward PBLH side chains. In fact $HClO_4$, H_2SO_4, and HCl are practically indistinguishable since the experimental points fit on the same curve; TFA behaves like $HClO_4$, H_2SO_4, and HCl until ratios close to 0.2, and then it exhibits a slightly lower protonating power. At high degrees of protonation the electrostatic repulsion among positively charged side chains makes the proton addition more and more difficult as the extent of protonation increases. As a consequence,

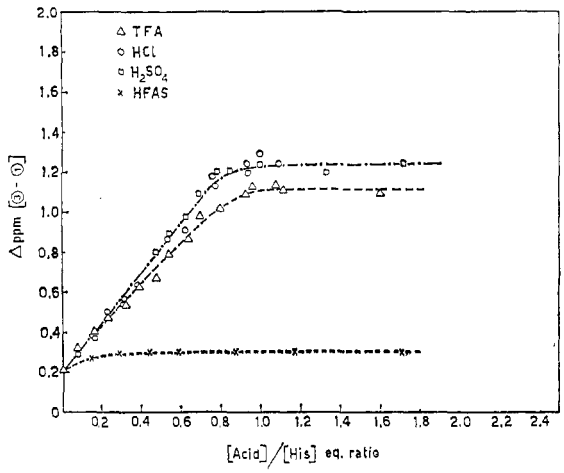

Figure 2. Relative chemical shifts of the imidazole protons (C-3) with respect to the phenyl protons (1) as a function of the [acid]/[His] equivalent ratio. The data obtained with $HClO_4$ in our previous work[1] overlap those obtained with HCl.

the maximum extent of protonation, that is, the chemical shift corresponding to the plateau region of Figure 2, depends on the strength of the acid. If we assume that in the presence of HCl or H_2SO_4 the degree of protonation of the polymer side chains does correspond to the [acid]/[His] equivalent ratio for ratios lower than 0.7, it is possible to calculate the theoretical chemical shift corresponding to 100% protonation. This can be done by extrapolating the linearly increasing portion of the graph corresponding to HCl (or H_2SO_4) of Figure 2 to [HCl]/[His] = 1. From the extrapolated value the extent of protonation of PBLH side chains in the plateau region of Figure 2 for the various acids can be roughly estimated. It turns out that at [acid]/[His] ratios between 1 and 2 the extent of protonation of the 1-benzylimidazole side chains is *ca.* 84% for HCl and H_2SO_4, *ca.* 74% for TFA, and *ca.* 8% for HFAS. These figures of course are valid when the polymer concentration is 8% (w/v). The low extent of protonation observed in the presence of HFAS indicates that we are dealing with a very weak acid. In this case the equilibrium PBLH + HFAS $\rightleftarrows$ PBLH·HFAS should not be substantially influenced by the "polyelectrolyte effect" since the charged side chain groups are far enough from each other.

If we look at the nmr spectra of PBLH (Figures 1a–d), we observe that in the presence of H_2SO_4, HCl, and TFA the peaks become more and more sharp as the [acid]/[His] ratio increases from 0 to *ca.* 1. On the other hand, no change of the peak shapes is observed when HFAS is added to the polymer solution. This point will be discussed later in connection with the conformational properties of PBLH in the presence of the various acids.

CD Properties of PBLH in TFE in the Presence of Various Acids. As reported in the Experimental Section, varying amounts of 1 *N* solutions of H_2SO_4, HCl, TFA, and HFAS were added to a PBLH solution in TFE. Since the maximum polymer concentration was 0.5 g/l. and the maximum [acid]/[His] ratio was 2, a simple calculation shows that the maximum water content of the polymer solutions was 0.2% (w/v). Some preliminary CD measurements showed that this amount of H_2O has no effect on the CD spectrum of PBLH in TFE.

Figure 3 shows some typical CD spectra of PBLH in the presence of varying amounts of H_2SO_4. The spectrum recorded in the presence of the stoichiometric amount of $HClO_4$ (with respect to the histidine residues) is also reported for

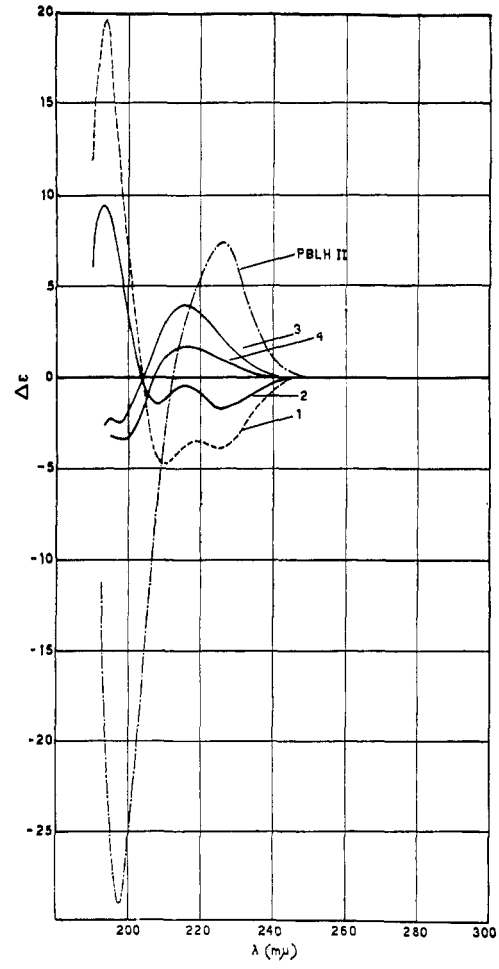

Figure 3. Some typical CD spectra of PBLH in TFE at various $[H_2SO_4]$/[His] equivalent ratios: 1, 0 (no added acid); 2, 0.40; 3, 1.00; 4, 2.00. The spectrum of PBLH II obtained in the presence of the equivalent amount of $HClO_4$ is also reported for comparison.

comparison. According to our previous investigation,[1] the spectrum in pure TFE has been interpreted as corresponding to that of the right-handed α-helical conformation. Increasing amounts of H_2SO_4 cause a marked change in the CD pattern; a new positive, broad band is formed, located at 216 mμ, followed by a negative one whose minimum is located below 200 mμ. When the amount of added acid is stoichiometrically equivalent to the histidine residues (*i.e.*, at an [acid]/[His] ratio of 1), the Δε value of the positive band is 4.1. On increasing the acid content further the positive band diminishes until [acid]/[His] ratios of *ca.* 2, and then it remains constant. The final Δε value of the positive band is 1.5. On the other hand, in the same range of acid content, the intensity of the negative portion of the spectrum increases slightly.

The results reported in Figure 3 clearly indicate that H_2SO_4 does not induce the same polymer conformation as $HClO_4$. The effects of HCl and TFA on the CD spectrum of PBLH are very similar to that of H_2SO_4 (Figure 4). These acids cause the progressive disappearance of the typical spectrum of the right-handed α-helix and the formation of a CD pattern having almost the same shape as that obtained in the presence of H_2SO_4. There is a positive band at 216 mμ, a shoulder around 210 mμ, and the beginning of a negative band located below 190 mμ. We note that in this case the negative portion of the spectrum appears blue shifted with respect to that observed with H_2SO_4. The spectrum reaches its final form at [acid]/[His] ratios near 1. Under such conditions

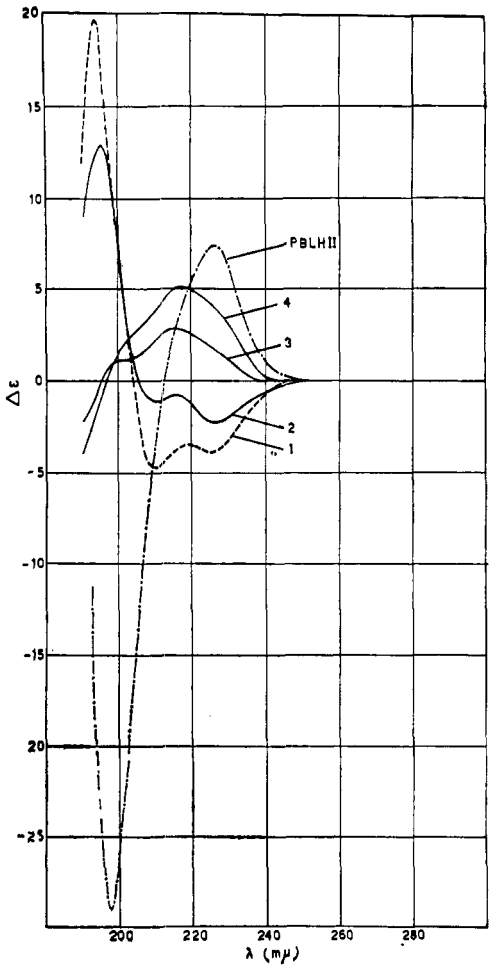

Figure 4. Some typical CD spectra of PBLH in TFE, at various [HCl]/[His] equivalent ratios: 1, 0 (no added acid); 2, 0.38; 3, 0.61; 4, 1.00. The spectrum of PBLH II is also reported for comparison.

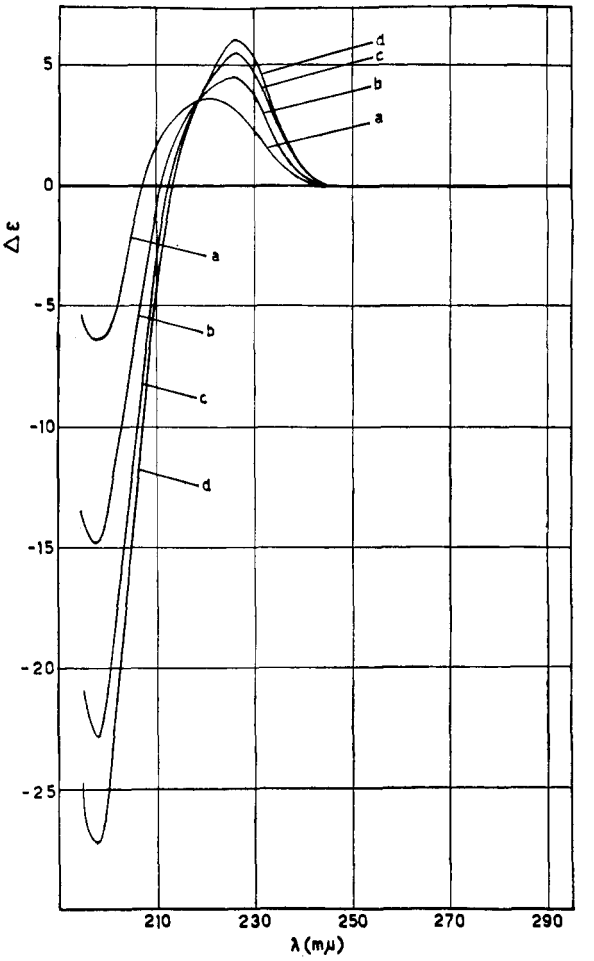

Figure 6. Some typical CD spectra of PBLH·HCl in TFE at various [ClO_4^-]/[His] equivalent ratios: a, 0.20; b, 0.32; c, 0.45; d, 0.70.

the $\Delta\epsilon$ value at 216 mμ is 5.1. Neither the shape of the whole CD pattern nor the intensity of the bands changes on further increasing the HCl or TFA content, at least until [acid]/[His] ratios near 2. Also in this case a substantial difference with respect to the data obtained with $HClO_4$ is clearly evident.

When HFAS is used as protonating agent no effect on the CD pattern has been detected until [HFAS]/[His] ratios of at least *ca.* 2.

Figure 5 summarizes the CD data obtained with the various acids; the $\Delta\epsilon$ values at 216 mμ are plotted as a function of the [acid]/[His] ratio. The results reported in this figure rule out the possibility that the observed variations of the CD patterns of PBLH in the presence of H_2SO_4, HCl, and TFA at [acid]/[His] ratios between 0 and 1 are merely due to the change of the nature of the side chain chromophores (because of protonation) without any change of the polymer conformation. In fact, if protonation were the only factor responsible for the variation of the CD pattern we should observe a gradual variation of the CD properties, very similar to that of the nmr spectra on increasing the [acid]/[His] ratio from 0 to 1. The S-shaped curves of Figure 5 clearly indicate that PBLH undergoes a conformational transition between 0.3 and 0.6 [acid]/[His] ratio.

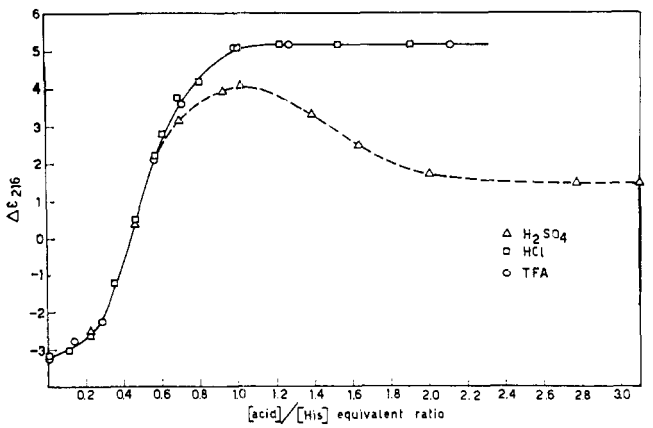

Figure 5. $\Delta\epsilon$ values at 216 mμ of PBLH in TFE reported as a function of the [acid]/[His] equivalent ratio.

Let us consider now in some detail the nature of the conformational transition occurring in the presence of H_2SO_4, HCl, and TFA. The CD spectrum of PBLH at [acid]/[His] = 1 is very much the same as that found in pure HFAS in the presence of the stoichiometric amount of $HClO_4$.[1] From CD studies on random copolymers of 1-benzyl-L-histidine with N^{ϵ}-carbobenzoxy-L-lysine in our previous paper,[1] the conclusion was drawn that protonated PBLH in HFAS is in the random coil conformation. On the basis of the similarities with the CD pattern of PBLH·$HClO_4$ in HFAS we conclude that the CD spectra of PBLH·$0.5H_2SO_4$, PBLH·HCl, and PBLH·TFA in TFE do correspond to a random coil conformation and that the transition shown in Figure 5 is a helix–coil transition.

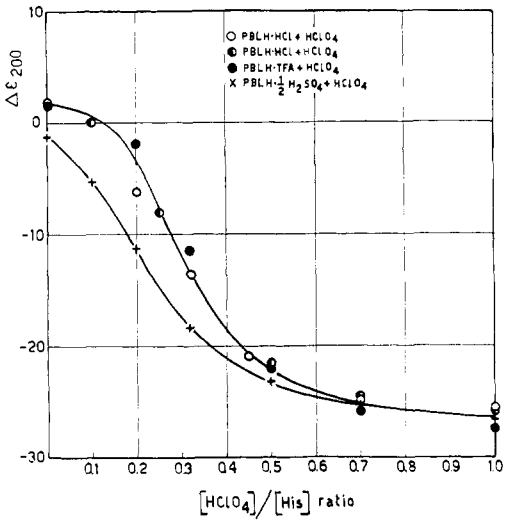

Figure 7. $\Delta\epsilon$ values at 200 mμ of protonated PBLH as a function of the $[ClO_4^-]$/[His] equivalent ratio. The symbol ◐ above refers to PBLH·HCl_4 + $LiClO_4$, rather than $HClO_4$ as written.

It is important to remark that H_2SO_4, HCl, and TFA are indistinguishable with respect to their effect on the conformation of PBLH in TFE (see Figure 5) at least for [acid]/[His] ratios lower than 0.7. These findings are consistent with the almost identical protonating power of the acids shown by nmr measurements (see Figure 2). The effect of H_2SO_4 on the intensity of the CD band at 216 mμ is slightly different from that of HCl and TFA at [acid]/[His] ratios higher than 0.7, and deserves further comment. We observed that the CD band at 216 mμ reaches its maximum intensity at [acid]/[His] = 1, then slowly diminishes and reaches a constant value at and [acid]/[His] ratio equal to 2. A possible explanation for this effect could be the following. When the acid content is equal to the stoichiometrically equivalent amount with respect to the histidine residues, the side chain groups of the polymer are nearly fully protonated. Since both protons of H_2SO_4 have been used, sulfate ions are present. Higher amounts of acid should produce bisulfate ions and it is possible that a specific binding of HSO_4^- ions to the protonated 1-benzylimidazole groups is responsible for the diminished intensity of the 216-mμ CD band. Such a hypothesis of course implies that this band contains contributions from the protonated side chain chromophores. It has been already remarked that the 216-mμ band is quite strong and very broad, and probably arises from overlapping of more than one band. Since the contribution from the amide chromophores is known to be small in this spectral region for coiled peptides,[4] it is clear that there are contributions from the protonated 1-benzyl-L-imidazole groups to the 216-mμ band. It seems, therefore, not unlikely that specific binding of ions may affect the rotational strength of the electronic transitions of such groups and therefore the CD pattern of the random coil form.

All the above results show that PBLH·HCl, PBLH·0.5-H_2SO_4, and PBLH·TFA do not assume the conformation of PBLH II in TFE. Such a conformation has been obtained in the presence of $HClO_4$. In order to make more evident the effect of this acid, the following experiments have been performed. To a solution of PBLH in TFE containing an equivalent amount of HCl, increasing proportions of $HClO_4$ have been added. The corresponding CD spectra indicate

(4) S. N. Timasheff and M. G. Gorbunoff, *Annu. Rev. Biochem.*, **36**, 13 (1967).

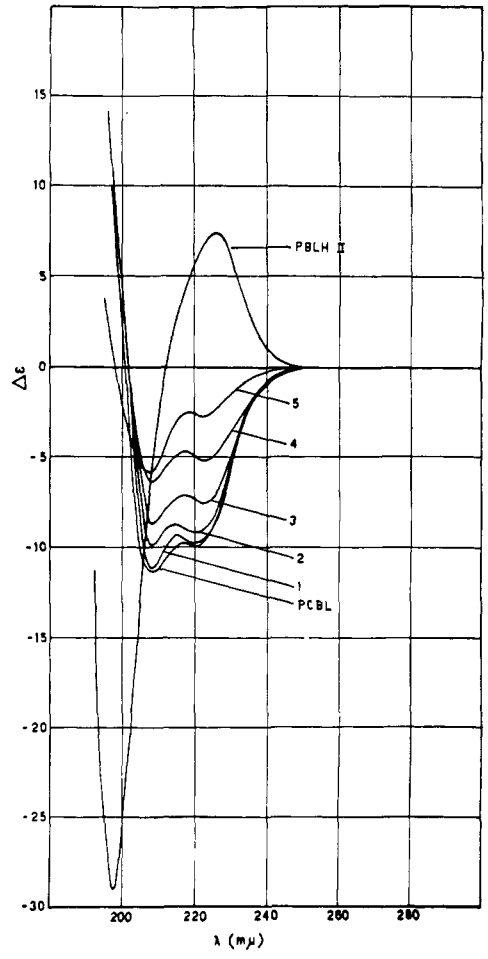

Figure 8. CD spectra in TFE of random copolymers of 1-benzyl-L-histidine and N^{ϵ}-carbobenzoxy-L-lysine, containing the stoichiometric amount of $HClO_4$ with respect to histidine residues. The numbers on the curves refer to the copolymer compositions given in Table I of our previous paper.[1]

that $HClO_4$ causes the formation of PBLH II (Figure 6). The same result has been obtained by using PBLH·TFA or PBLH·0.5H_2SO_4, and adding either $HClO_4$ or $LiClO_4$. The results of such experiments are summarized in Figure 7, where the $\Delta\epsilon$ values of protonated PBLH at 200 mμ are plotted as a function of the $[ClO_4^-]$/[His] ratio. There is evidence for a conformational change which corresponds to a *random coil form II* transition induced by the presence of ClO_4^-. Also in this case the experimental points obtained with PBLH·HCl fall on the same curve as those obtained with PBLH·TFA, while PBLH·0.5H_2SO_4 behaves in a slightly different way. This behavior is consistent with the CD pattern of PBLH·0.5H_2SO_4, which is slightly different, especially in the negative portion, from that of PBLH·HCl or PBLH·TFA, and with our previous hypothesis concerning bisulfate ion formation. In fact, with PBLH·0.5H_2SO_4 it is possible that the addition of $HClO_4$ causes bisulfate ions to be formed, which should enhance the intensity of the negative portion of the CD spectrum. This could explain why the curve corresponding to the coil form II transition for PBLH·0.5H_2SO_4 falls below the curve of PBLH·HCl and PBLH·TFA.

CD Properties of Random Copolymers of 1-Benzyl-L-histidine and N^{ϵ}-Carbobenzoxy-L-lysine. Figures 8 and 9 show the CD spectra of random copolymers of 1-benzyl-L-histidine and N^{ϵ}-carbobenzoxy-L-lysine in TFE. Each copolymer solution contained an exactly equivalent amount

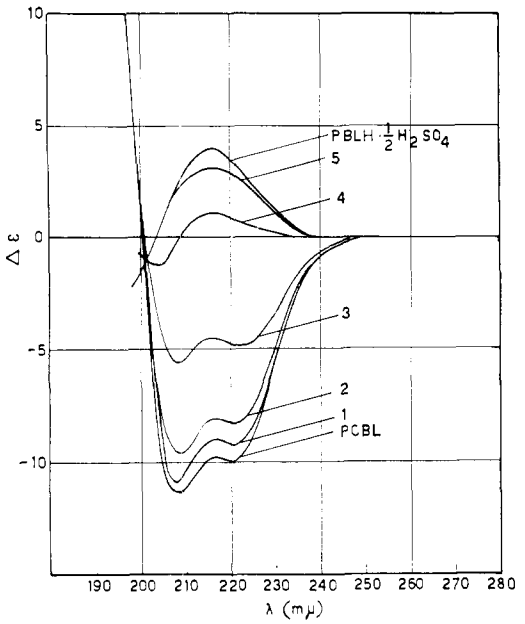

Figure 9. CD spectra in TFE of random copolymers of 1-benzyl-L-histidine and N^{ϵ}-carbobenzoxy-L-lysine containing the equivalent amount of H_2SO_4 with respect to histidine residues. The numbers on the curves refer to the copolymer compositions given in Table I of our previous paper.[1]

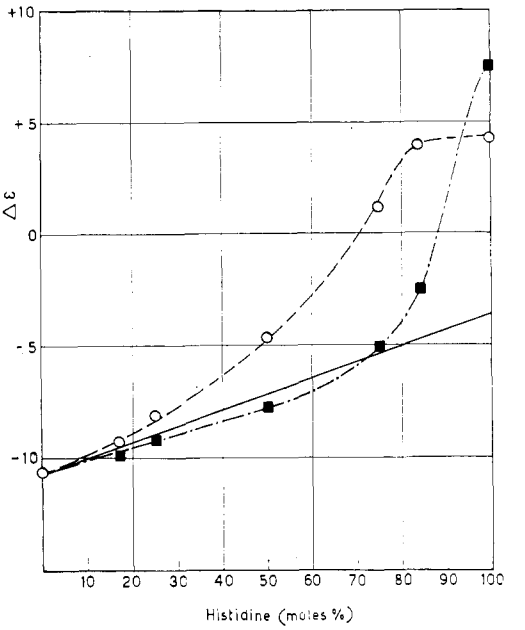

Figure 10. $\Delta\epsilon$ values at fixed wavelengths for random copolymers of 1-benzyl-L-histidine and N^{ϵ}-carbobenzoxy-L-lysine in TFE as a function of the copolymer composition. All copolymers contain the equivalent amount of $HClO_4$ (-··■··-) or H_2SO_4 (---○---). In the case of protonation with $HClO_4$ the $\Delta\epsilon$ values have been measured at 226 mμ; in the case of protonation with H_2SO_4 the $\Delta\epsilon$ values have been measured at 216 mμ. The solid line represents the $\Delta\epsilon$ values at 220 mμ in absence of any added acid, from our previous work.[1]

of $HClO_4$ and H_2SO_4, respectively, with respect to the histidine residues. The spectrum of pure poly(N^{ϵ}-carbobenzoxy-L-lysine) (PCBL) is reported for comparison. When the basic side chain groups of the copolymers are neutralized with $HClO_4$, the typical CD pattern of PBLH II begins to appear at high histidine contents in the copolymer chains. When H_2SO_4 is used as protonating agent, the CD spectrum of the coiled form becomes more and more evident as the histidine content of the copolymers is increased.

It is evident from Figures 8 and 9 that the CD spectra do not change in a gradual fashion on increasing the histidine content of the copolymers. This is more evident in Figure 10, where the $\Delta\epsilon$ values at 226 mμ for the case of $HClO_4$, and at 216 mμ for the case of H_2SO_4, are reported as a function of the copolymer composition. The solid line represents the behavior of the CD spectrum ($\Delta\epsilon$ at 220 mμ) as a function of the copolymer composition in absence of any added acid.[1] In both cases, there is evidence for a conformational transition of the peptide chains on going from pure PCBL to protonated PBLH. This behavior is expected when the conformations of the two pure homopolymers are different.[5-10] In our case the conformations of PBLH·$HClO_4$ (PBLH II) and of PBLH·$0.5H_2SO_4$ are different from the right-handed α-helical conformation of pure PCBL.

Conclusions

The data presented in this work allow us to conclude that protonated PBLH in TFE assumes different conformations when different acids are used as protonating agents; the ordered conformation of PBLH II can be obtained only in the presence of $HClO_4$. Other acids such as H_2SO_4, HCl, and TFA cause the appearance of the random coil conformation. Finally, HFAS, which is a very weak acid, does not alter the α-helical form, at least in the range of [HFAS]/[His] ratios examined. Substantially higher amounts of HFAS in the solvent mixture are expected to induce a helix–random coil transition, since in pure HFAS the polymer has been shown to be in the coiled form.[1]

On the whole, these results lead to the conclusion that protonation of the side chain groups of PBLH in TFE is not a sufficient condition to stabilize PBLH II. In this respect the presence of ClO_4^- ions seems to be of importance. A possible explanation for the effect of $HClO_4$ could rest on a specific binding of ClO_4^- ions to the protonated 1-benzylimidazole groups of PBLH. Such an effect is not completely surprising since ClO_4^- ions have substantial effects also on the conformation of protonated poly(L-lysine) and of random copolymers of L-lysine with L-phenylalanine in aqueous solution.[11]

In light of the above conclusions it is possible to offer an explanation for the progressive sharpening of the nmr peaks of the side chain groups of PBLH on increasing the amounts of H_2SO_4, HCl, and TFA. In pure TFE the side chain groups are "frozen" in the rigid α-helical conformation. Their motion is prevented, with consequent broadening of the nmr peaks.[12] Increasing amounts of acids induce the random coil conformation, in which the side chain groups are much more free in their motions, with consequent sharpening of the nmr peaks. In the case of HFAS the absence of any change of shape of the nmr peaks is consistent with the

(5) R. M. Karlson, K. S. Norland, G. D. Fasman, and E. R. Blout, *J. Amer. Chem. Soc.*, **82**, 2268 (1960).
(6) M. Hashimoto and J. Aritome, *Bull. Chem. Soc. Jap.*, **39**, 2707 (1966).
(7) M. Hashimoto, *ibid.*, **39**, 2713 (1966).
(8) M. Goodman, A. M. Felix, C. M. Deber, A. R. Brause, and G. Schwartz, *Biopolymers*, **1**, 371 (1963).
(9) E. Peggion, A. S. Verdini, A. Cosani, and E. Scoffone, *Macromolecules*, **2**, 170 (1969).
(10) E. Peggion, A. Cosani, A. S. Verdini, A. Del Pra, and M. Mammi, *Biopolymers*, **6**, 1477 (1968).
(11) E. Peggion, *et al.*, in preparation.
(12) F. J. Joubert, N. Lotan, and H. A. Scheraga, *Biochemistry*, **9**, 2197 (1970).

absence of any effect of this weak acid on the polymer conformation.

In this connection it should be recalled that $HClO_4$ also causes a progressive sharpening of the nmr peaks of the side chain groups of PBLH in TFE.[1] This fact leads to the conclusion that the side chain groups are also quite free in their motion when the polymer is in the conformation of PBLH II.

More detailed studies of the exact structure of PBLH II by X-ray diffraction methods and 220-MHz nmr spectroscopy are in progress in our laboratory and will be reported elsewhere.

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Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. I. Properties of Copolymers and Approximate Theories[1]

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ABSTRACT: Since it is necessary to have the Zimm-Bragg parameters σ and s of all naturally occurring amino acids (in water) for studies of protein conformation, and since these cannot be obtained from studies of the helix–coil transition in homopolymers, because of experimental difficulties, a technique has been developed to circumvent these problems. It involves the study of the thermally induced transition curves for random copolymers of "guest" amino acid residues in a water-soluble "host" poly(amino acid). The data may be interpreted with the aid of suitable theories for the helix–coil transition in random copolymers to obtain σ and s for the "guest" residues. While exact theories are available (for the one-dimensional nearest-neighbor Ising model), the computer costs involved in the computations are prohibitively high. Therefore, resort is had to approximate theories. It is shown here that, for the usual ranges of parameters found for poly(amino acids), one of the two lowest order approximations (corresponding to earlier treatments by Lifson and Allegra) is completely adequate, *i.e.*, gives results essentially identical with the exact result. In essence, the low-order approximations hold if σ and s for the two constituents of the copolymer do not differ appreciably from each other. If they do, then higher order approximations (which become exact in highest order) are required. Calculations are also reported for both regular-sequence and random copolymers in order to demonstrate how the amino acid sequence and composition of the copolymer, and also the values of σ and s of the constituents, affect the transition temperature, the breadth of the transition, and the most probable length of a helical sequence in the copolymers. It is shown that small departures from a random sequence do not affect the melting curve significantly.

Since near-neighbor interactions play a very important role in determining the conformation of an amino acid residue in a protein,[3,4] it is desirable to have a quantitative measure of the relative stabilities of the α-helix and random-coil conformations of each naturally occurring amino acid in water. Such data would then provide a basis to compute the propensity for any given sequence of amino acids in a protein to adopt the α-helical conformation.[5] In principle, these data can be obtained by measuring the fraction of α helix for each homopoly(amino acid) as a function of chain length and temperature, and interpreting the experimental results, for example, by the Zimm–Bragg[6] theory. For such an approach to be experimentally feasible, the homopoly(amino acid) must be water soluble, α helical, and capable of being melted in the temperature range between 0 and 100°C. Unfortunately, none of the homopolymers of the naturally occurring amino acids satisfies all three of these requirements. While the use of block copolymers[7-12] solves the solubility problem for any amino acid, it circumvents the other two difficulties only for a few amino acids; hence, a technique (the subject of this series of papers) has been developed which is applicable to *any* amino acid. It involves the study of random copolymers in which the desired amino acid, the "guest," is incorporated at random into a nonionic homopoly(amino acid), the "host," which does meet all three requirements. The helix–coil stability constants of the guest residue can then be determined from its influence on the melting behavior of the host homopoly(amino acid).

In this first paper of the series, we will examine the ways in which the melting behavior of a copolymer of a given composition differs from that of a homopolymer, focusing atten-

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