Biophysical Chemistry 25 (1986) 127–134
Elsevier

BPC 01096

CONFORMATIONAL STUDY OF TWO SYNTHETIC PEPTIDES WITH SEQUENCE ANALOGIES TO THE N-TERMINAL FRAGMENT OF RNase A

A. BIERZYŃSKI [a], M. DADLEZ [a], M. SOBOCIŃSKA [b] and G. KUPRYSZEWSKI [b]

[a] *Institute of Biochemistry and Biophysics, Polish Academy of Sciences, ul. Rakowiecka 36, 02-532 Warszawa and* [b] *Institute of Chemistry, University of Gdańsk, ul. Sobieskiegoi 18, 80-952, Gdańsk, Poland*

Received 25th June 1986
Accepted 5th September 1986

Key words: α-Helix; C-peptide conformation; Salt bridge

The conformational properties of two synthetic model peptides, AEAAHAAEAAHMG (PA) and AEAAHAFEAAHMG (PF), have been studied using CD and ^{1}H-NMR methods. In both peptides, glutamate and histidine residues are situated in such a way that two salt bridges between Glu^- (i) and His^+ $(i+3)$ can be formed. A salt bridge of this type (Glu^- 9-His^+ 12) was postulated previously to stabilize, to a great extent, the α-helical conformation of isolated N-terminal fragments of RNase A: C-peptide and S-peptide (A. Bierzyński, P.S. Kim and R.L. Baldwin, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 2470). Although in both PA and PF salt bridges between glutamates and histidines are formed, as demonstrated by the pH-titration curves of the glutamate γ-proton signals, no traces of helical conformation have been detected. Evidently, the Glu^- (i)-His^+ $(i+3)$ salt bridges do not stabilize the α-helical conformation. A comparative analysis of PA and PF NMR spectra provides strong evidence that the phenylalanyl ring in PF interacts not only with the hydrophobic methyl groups of almost all alanine residues but also with the histidine rings and the glutamate side chains in their protonated as well as deprotonated forms. Similar interactions, involving Phe 8, can be expected in the N-terminal fragments of RNase and should be taken into account as an important factor determining the conformational properties of C- and S-peptides.

1. Introduction

The ability of some polypeptide chains to adopt the α-helical conformation in water solutions has been attracting the interest of many biophysicists for a long time. In the last three decades an impressive number of theoretical and experimental works on this subject have been published [1a]. Nevertheless, the basic question, namely, how helix stability depends on a peptide sequence, still remains unanswered. In the light of recent studies on the conformation of isolated N-terminal fragments of RNase A, C-peptide [1b–4] and S-peptide [2,5–7], it seems certain that some specific interactions between amino acid side chains are one of the main factors promoting stability of the helix or destabilizing it. However, very little is known about these interactions, as yet.

In our laboratory a series of works has been undertaken on the conformational properties of a number of model peptides and analogues of N-terminal fragments of RNase A, with the aim of elucidating the possible role of some side chain-side chain interactions in α-helix formation by isolated C-peptide in water solutions. This paper is the first of the series.

It has been postulated that one of the main contributions to α-helix stability in C-peptide [1] and S-peptide [6] is provided by a salt bridge between Glu^- 9 and His^+ 12 side chains (fig. 1).

We have decided to examine this hypothesis through an investigation of a model peptide (PA) containing two histidines and two glutamates situated in an appropriate way, so that two Glu^- (i)-His^+ $(i+3)$ salt bridges could be formed. Alanine residues, known as good helix makers [8,9], where chosen as spacers. The peptide sequence is shown in fig. 1. Besides the two salt

```
PA    AEAAHAAEAAHMG

SP  KETAAAKFERQHMDSSTSA

PF    AEAAHAFEAAHMG


PAL   AEAAHAAEAAHHse (lactone)

CPL KETAAAKFERQHHse (lactone)

PFL   AEAAHAFEAAHHse (lactone)
```

Fig. 1. Sequences of the peptides studied in this work compared with those of C-peptide lactone (CPL) and S-peptide (SP) of RNase A. The expected salt bridges stabilizing the α-helix are indicated in the PA sequence.

bridges, Glu^- 2-His^+ 5 and Glu^- 8-His^+ 11, an alternative salt bridge, His^+ 5-Glu^- 8, is also possible and should further stabilize the helical conformation [10]. Consequently, there is every reason to suppose that peptide PA should be predominantly helical, at favorable pH, if the salt bridge hypothesis is true.

The only destabilizing effect may be expected from the presence of the negatively charged C-terminal α-carboxyl group of Gly 13. A similar group is known to destabilize the helix in C-peptide carboxylate [2,3]. Therefore, we have also obtained, for comparison, the CNBr-cleaved fragments of PA (fig. 1) with the C-terminal homoserine residue either in the lactone (PAC) or carboxylate (PAC) form as in the respective forms of C-peptide.

We were also interested as to what conformational effects would be related with the introduction of a phenylalanine residue into the PA sequence. We have synthesized the Ala 7 → Phe analogue of PA (PF) in which the phenylalanine residue is situated at the same position, relative to Ala 3, Ala 4, Glu 8, His 11 and Met 12 as in the S-peptide sequence Phe 8 is situated relative to the Ala 4, Ala 5, Glu 9, His 12 and Met 13 residues (fig. 1).

For the same reasons as in the case of PA, the CNBr fragments of PF (PFL and PFC) have also been obtained (fig. 1).

2. Materials and methods

2.1. Peptide synthesis

The peptides PA and PF were synthesized by the solid-phase method [11,12] on a Beckman model 990 peptide synthesizer. The first Boc-amino acid was coupled to chloromethyl polystyrene resin (capacity of Cl = 196 μM/g) using caesium salt. For the synthesis, the α-amino groups were protected with *t*-butyloxycarbonyl (Boc) and the side chain-protecting groups were as follows: benzyl ester for the carboxyl group of glutamate and *p*-toluenesulphonyl for the imidazole of histidine. Trifluoroacetic acid (33% in CH_2Cl_2) with 2% dimethyl thioether as a scavenger was used to remove the Boc, and the resulting salt was neutralized with trimethylamine (10% in CH_2Cl_2). Dicyclohexylcarbodiimide (DCC) was used to couple the Boc-amino acids. The protecting groups were removed and the peptides were cleaved from the resin at 0°C with anhydrous HF containing 10% anisole as a scavenger. The peptides were washed out from the mixture after completion of the reaction with 10% acetic acid and then lyophilized.

The peptides were purified on Sephadex G-15 or G-25 columns. Final purification was carried out on a Sephadex LH-20 column. The peptides were chromatographically homogeneous (TLC) and gave the required amino acid composition in amino acid analysis.

The NMR spectra of the peptides showed no traces of any impurities, but the presence of a sharp singlet at 2.71 ppm indicated that Met 12 had been partly oxidized, in both, to methionine sulphoxide. The sulphoxide was reduced by incubation for 52 h in 3 M mercaptoethanol in unbuffered water, 37°C [13].

The CNBr cleavage [14] of the peptides PA and PF to PAL and PFL (fig. 1), respectively, was carried out at room temperature in 70% formic acid. The peptide concentration was 10 mg/ml and an 80-fold excess of CNBr was used. After 24 h the reaction mixture was diluted 10-fold with water and freeze-dried. The product was dissolved in 0.1 M HCl and kept for at least 3 days, at room temperature, for full conversion of Hse 12 into its lactone form. Ion-exchange chromatography on

an SP-Sephadex C-25 column run in 20 mM cacodylate buffer, pH 5.4, using an NaCl gradient of 0–0.5 M, proved that the material was indeed 100% lactone. It was desalted on a Sephadex G-10 column run in 10 mM HCl and lyophilized. When necessary, PAL and PFL were converted into their respective carboxylic forms, PAC and PFC, by keeping them for at least 24 h at pH 9, at room temperature.

2.2. CD spectra

CD spectra were recorded on a Jasco J-20 spectropolarimeter with a thermostatted cell holder. All samples contained 0.1 M NaCl/1 mM citrate/1 mM sodium phosphate/1 mM sodium borate and the pH was adjusted with concentrated HCl or NaOH. Peptide concentration, determined by weight of dry material, was kept, as a rule, at 0.05 mg/ml and the cell path length was 1.0 cm. The same spectra were obtained when 10-fold greater concentrations and cells with a path length of 1.0 mm were used. These latter conditions were always used for samples containing 6 M urea. Analytical grade urea was crystallized from ethanol and dried under vacuum.

2.3. Ultraviolet absorption

Ultraviolet absorption at 197 nm was measured on a Cary 118 spectrophotometer equipped with a thermostatted cell holder. All samples contained 0.1 M NaF and a minute amount of HCl to maintain the pH at about 5.

2.4. NMR

Fourier-transform proton spectra were recorded on a Bruker 270-MHz instrument in the Department de Chimie Organique, UER des Sciences Pharmaceutiques et Biologiques, Universite Rene Descartes Paris V. For PF, some measurements were repeated on a Bruker AM-500 apparatus in the Max-Planck-Institut fur Medizinische Forschung in Heidelberg. All samples were prepared in ^2H_2O containing 0.1 M NaCl without buffer. p^2H was adjusted by adding concentrated solutions of ^2HCl or NaO^2H. The pH-meter readings were not corrected for isotope effects [15]. TSP was used as an internal reference. At $p^2H > 3$ corrections were made for the pH dependence of the TSP chemical shift [16].

Peptide concentrations were 6 mM or less. No change in the spectra was observed when the concentration was reduced 2-fold. The spectra were recorded at 5°C, 25°C, and occasionally at higher temperatures up to 50°C. Reported results refer to the temperature of 25°C, if not stated otherwise.

2.5. Calculation of pH-titration curves

In general, a molecule can contain n titratable groups with dissociation constants K_1, $K_2 \ldots K_n$. Assuming that the Hill coefficient equals 1 [17] the fraction of molecules with group i in protonated form is $h_i = [H^+]/([H^+] + K_i)$. There are 2^n different protonation states of the molecule. The molar fraction of each state is given by the product $\Pi_{i=1}^{n} x_i$ where $x_i = h_i$ or $1 - h_i$. When the observed chemical shift depends on the state of protonation of only one group

$$\delta = \delta_h h + \delta_d (1 - h) \tag{1}$$

where δ_h and δ_d are the chemical shifts for the protonated and deprotonated state, respectively. In the case when δ depends on two groups.

$$\delta = \delta_{hh} h_1 h_2 + \delta_{hd} h_1 (1 - h_2) + \delta_{dh} (1 - h_1) h_2 + \delta_{dd} (1 - h_1)(1 - h_2) \tag{2}$$

where δ_{hh}, δ_{hd}, δ_{dh} and δ_{dd} are the respective chemical shifts for each of the four possible states of the molecule.

If $K_1 \gg K_2$, as for Glu and His^+, $h_1(1 - h_2) \approx 0$ and with an excellent approximation we may put either $\delta_{hd} = \delta_{hh}$ or $\delta_{hd} = \delta_{dd}$, depending on whether δ is more sensitive to the protonation of the first or of the second group. Therefore, from eq. 2 we obtain two alternative expressions:

$$\delta = \delta_{hh} h_1 + \delta_{dh} (1 - h_1) h_2 + \delta_{dd} (1 - h_1)(1 - h_2) \tag{3}$$

$$\delta = \delta_{hh} h_1 h_2 + \delta_{dh} (1 - h_1) h_2 + \delta_{dd} (1 - h_2). \tag{4}$$

The functions defined by eqs. 1, 3 or 4 were fitted to observed NMR chemical shifts using a least-squares iterative procedure.

3. Results

3.1. CD and ultraviolet measurements

In analogy to C-peptide, we expected that from all the peptides we studied, PAL and PFL would show the highest propensity for α-helix formation at low temperature and pH around 5. The CD spectra of PAL at various temperatures and pH 5 are presented in fig. 2. The spectrum measured at 6°C does not show any traces of the α-helical conformation and remains unchanged in the presence of 6 M urea. A similar CD spectrum was reported for C-peptide in 5 M guanidine hydrochloride [18]. Apparently, at low temperatures the conformation of PAL is close to the random coil. With increasing temperature, the CD spectra change noticeably (fig. 2) indicating that a partial transition to an ordered conformation takes place. The observed changes in CD spectra cannot be accounted for by α-helix formation since they are related with a concomitant small increase in ultraviolet absorption at 197 nm (fig. 3) and just the opposite effect should be observed if the helix were formed [19]. An increase in absorption at 197 nm is consistent rather with a partial transition to some conformation of β-type [19] and the observed changes in the CD spectra do not exclude this possibility [20].

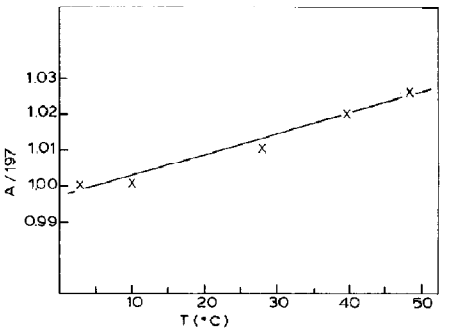

Fig. 3. Ultraviolet absorption at 197 nm of PAL in 0.1 M NaF at pH 5.0 as a function of temperature. Relative values with corrections for temperature expansions are presented.

In any case, the ordered conformation of the peptide is stabilized mainly by hydrophobic interactions as indicated by the temperature dependence of its content. It is certainly not related with any salt bridge formation since protonation of the carboxyl groups has no effect on its stability: the CD spectra of PA at pH 2.2 (not shown) are, independently of temperature, exactly the same as those measured at pH 5 (fig. 2). On the other hand, deprotonation of the histidine rings seems to enhance the structure slightly. For example, the spectrum measured at 27°C and pH 8.0 (not shown) closely resembles that at pH 5.0 and 47°C (fig. 2, curve 3) although at 6°C no differences between the spectra measured at pH 5.0 and 8.0 have been observed. Most probably, in basic solutions the ordered conformation of the peptide is further stabilized by the highly hydrophobic, deprotonated imidazole rings of His 5 and 11.

Introduction of phenylalanine into the peptide sequence does not result in any CD-detectable conformational changes. The spectra of PFL (not shown), independently of pH and temperature, are, within the error limits, the same as those of PAL (fig. 2). Chemical modifications of the C-terminus also seem to have no effect on peptide conformation: the CD spectra of PAC and PA are indistinguishable from those of PAL.

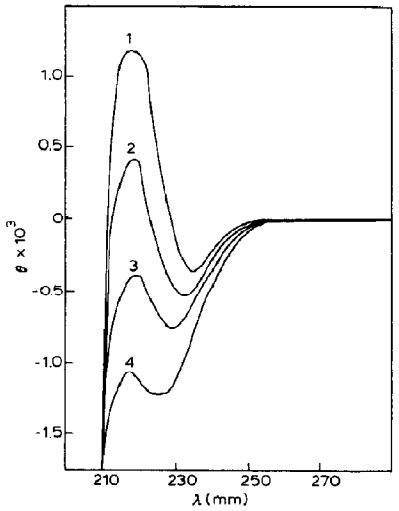

Fig. 2. CD spectra of PAL in 0.1 M NaCl, at pH 5.0; curve 1, 6°C; 2, 27°C, 3, 47°C; 4, 68°C.

3.2. NMR measurements

The temperature-induced conformational transitions of PA and PF observed in CD are not visible in proton NMR. For both peptides we could not observe any meaningful temperature shifts of the NMR proton signals. On the other hand, deprotonation of the histidines (pK 6.8) is monitored by changes in the methyl proton signals of four alanine residues in both PA and PF (fig. 4). The pH-titration curves of the alanine β-protons at 25°C (fig. 4) are very similar to those obtained at 5°C (not shown), although at low temperature no pH-induced conformational changes have been observed in CD. Most probably, then, the pH shifts of the alanine β-proton signals do not reflect a change in PA and PF backbone conformations but rather result from an interaction of the alanine methyl groups with the highly hydrophobic, deprotonated histidine rings.

pH titration of the glutamate γ-proton signals was of particular interest for us because it could be expected to provide an indication of whether the Glu^--His^+ salt bridges are formed in PA and/or PF. The pH-titration curves of these signals are shown in fig. 5. In PA, the γ groups of both Glu 2 and Glu 8 are indistinguishable from each other, but in PF the signal of one of them is shifted upfield by as much as 0.1 ppm. We believe that this is the signal of Glu 8, since this residue is the closest neighbour of Phe 7 in the PF sequence.

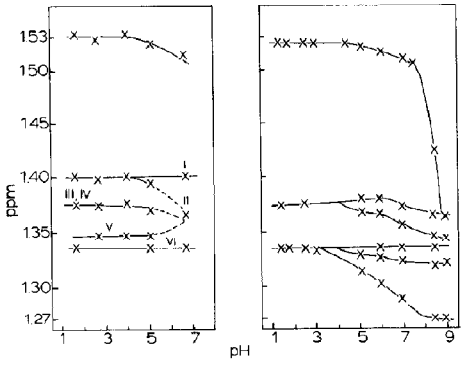

Fig. 4. pH titration of alanine β-proton signals of PA (left) and PF (right) in 0.1 M NaCl, at 25°C. Approximate positions of overlapping signals are indicated by broken lines.

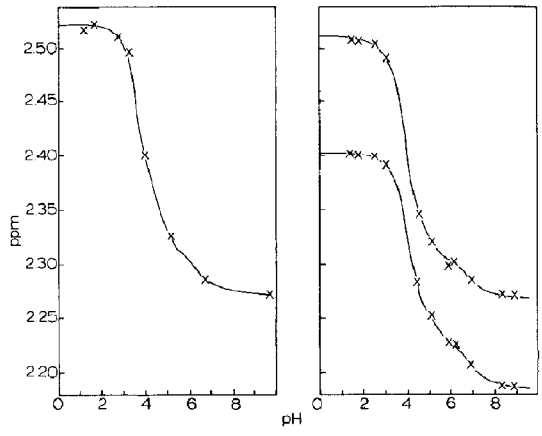

Fig. 5. pH titration of glutamate γ-proton signals of PA (left) and PF (right) in 0.1 M NaCl, at 25°C. Interpolation curves have been calculated using eq. 3 and pK_2 values fixed at 6.72 and 6.87 for PA and PF, respectively (see table 2). The best-fit parameters of the curves are given in table 1.

It is characteristic of all the glutamate γ-proton signals that they not only titrate with the glutamate carboxyl groups (p$K \sim 4$) but also still shift upfield with pH increasing from 6 to 8 (fig. 5). Their position is therefore evidently dependent on the state of protonation of the histidine rings and can be described only by two-pK functions. The titration curves in fig. 5 have been calculated using eq. 3. The pK_2 parameters have been fixed at the values found previously for the pK values of histidine deprotonation in PA (6.72) and PF (6.87), respectively (see table 2). The other parameters, calculated by an iterative fitting procedure, are given in table 1.

Similarly to the glutamate γ-protons, the α-protons of Gly 13 in both PA and PF are sensitive to histidine deprotonation and their pH-titration curves have been calculated in the same way (fig. 6 and table 1).

It is rather unlikely that the upfield shifts of the glutamate γ- and glycine α-proton signals related with deprotonation of the histidine rings could result from changes in polypeptide backbone conformations of PA and PF. The γ groups are distant from the backbone and are not expected to be sensitive to its conformation. Besides, the pH-titration curves of the Glu γ- and Glu α-protons

Table 1

The best-fit parameters of pH titration of glutamate γ- and glycine α-proton signals in PA and PF calculated according to eq. 3 For glycine, the average chemical shifts (in ppm) of both α-protons are given.

	PA			PF		
	Glu 2-γ	Glu 8-γ	Gly 13-α	Glu 2-γ	Glu 8-γ	Gly 13-α
δ_{hh}	2.520	2.520	4.022	2.511	2.404	4.009
δ_{dh}	–	–	3.756	2.305	2.233	3.750
δ_{dd}	2.288	2.288	3.743	2.271	2.186	3.729
pK_1	3.9	3.9	3.551	3.92	4.08	3.46
pK_2 [a]	–	–	6.72	6.87	6.87	6.87

[a] Fixed at the pK values of histidine determined previously from pH titration of histidine ring proton signals (see table 2).

are virtually the same at 5 (not shown) and 25°C, although a small change in the backbone conformation related with the histidine deprotonation is visible in the CD spectra at 27°C but not at low temperature. Therefore, the observed shapes of the titration curves of the protons adjacent to the carboxyl groups are related, most probably, with salt bridge formation between these groups and the protonated imidazole rings of His^+ 5 and 11.

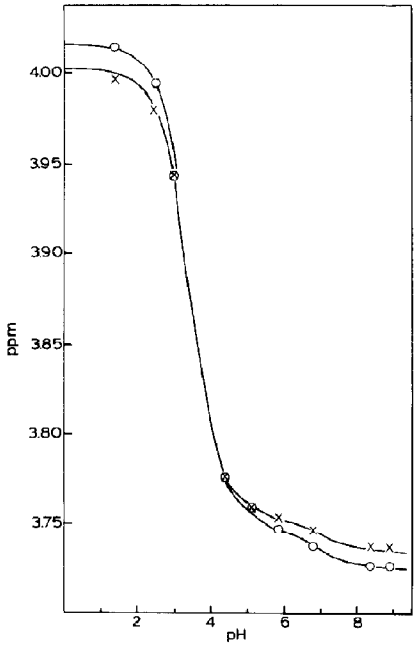

Fig. 6. pH titration of Gly 13 α-proton signals of PF in 0.1 M NaCl, at 25°C. The best-fit interpolation curves have been calculated using eq. 3, with the pK_2 value fixed at 6.87 (as for histidine). The same curves but shifted upfield by 0.014 ppm (not shown) have been obtained for PA. The titration parameters for both peptides are given in table 1.

The COO^--His^+ salt bridges are not visible in the pH-titration curves of the histidine ring proton signals. In PA as well as in PF the C(2)-H and C(4)-H signals of both histidines titrate normally, with the pK characteristic of the imidazole ring (see table 2). No shifts related with deprotonation of either the glutamate γ- or C-terminal carboxyl groups have been observed. Nevertheless, it cannot be taken as evidence against salt bridge formation because the histidine ring protons are rather insensitive to interactions with the carboxyl groups. In a model tripeptide, Gly-His^+-Gly-COO^-, in spite of a strong salt bridge between the His^+ ring and the COO^- group [2,21], the histidine ring proton signals do not shift more than 0.01 ppm upon protonation of the C-terminal carboxyl group.

The chemical shifts of many NMR signals are not the same in PA and PF. Although the alanine β-proton signals have not been assigned to definite residues, it is obvious (see fig. 4) that at least three are shifted upfield in PF by about 0.03 ppm at low pH. (One of these signals, viz. I, II, III or IV, may correspond to Ala 7 and be missing in PF.) In basic solutions yet another signal is shifted by 0.05 ppm. The γ-proton signals of Glu 2 are not affected by the presence of phenylalanine, but those of Glu 8 are strongly shifted upfield (~ 0.1 ppm). The ring proton signals of both histidines are also upfield-shifted in PF by a few hundredths of ppm (see table 2). A small but noticeable upfield shift (0.014 ppm) is visible for the Gly 13

Table 2

The best-fit parameters of pH titration of histidine ring proton signals in PA and PF calculated according to eq. 3

	C(2)-H			C(4)-H		
	PA	PF	Δ [a]	PA	PF	Δ [a]
δ_h (ppm)	8.646 8.634	8.617 8.587	0.038	7.329 7.315	7.274 7.260	0.055
δ_d (ppm)	7.675 7.670	7.647 7.669	0.015	6.954 6.954	6.933 6.885	0.045
pK	6.70 6.68	6.87 6.87		6.72 6.76	6.84 6.90	

[a] The average upfield shift of PF signals relative to PA.

α-protons (table 1). The observed shifts demonstrate that not only the hydrophobic groups of the alanine and deprotonated histidine residues, but also the highly polar carboxylate groups of Glu 8 and Gly 13 as well as the protonated rings of His 5 and 11 tend to keep close to the Phe 7 phenyl ring and are shielded by its π-electron system. Interestingly enough, the highly hydrophobic ϵ-methyl group of Met 12 does not interact with the Phe 7 ring. At low pH in both PA and PF its signal appears at the same position of 2.09 ppm and shifts upfield in the same way (0.01 ppm) upon histidine deprotonation.

Interactions of titratable groups with a phenylalanyl ring seem to lead to an increase of their pK values. The pK of the Glu 8 carboxyl group (table 1) as well as those of the His 5 and His 11 rings (table 2) are greater in PF than in PA (ΔpK = 0.15). On the other hand, the γ-carboxyl group of Glu 2, which does not interact with Phe 7, and the Gly 13 α-carboxyl group, for which a barely detectable interaction has been observed, titrate with the same pK values in both PA and PF (table 1).

4. Discussion

In none of the peptides that we studied has a trace of the α-helical conformation been detected. Nevertheless, the pH-titration curves of the glutamate γ-protons strongly suggest that the carboxyl groups of both glutamates do interact with the histidine rings. Apparently, Glu^- 2-His^+ 5 and Glu^- 8-His^+ 11 salt bridges are formed, but do not promote the helical conformation.

There is no reason to presume that the analogous salt bridge between Glu^- 9 and His^+ 12 in C-peptide can induce the helix. Therefore, the hypothesis about its α-helix stabilizing effect [1b] must be rejected. This conclusion has been corroborated by the observation of Shoemaker et al. [4] that substitution of Glu 9 by leucine in the C-peptide sequence leads not to a decrease, but rather to an increase of helix content.

Since residues i and $i \pm 3$ are close to each other in the α-helical structure, it is only natural to expect that an attraction between their side chains stabilize the helix. Nevertheless, it does not seem so obvious after a closer inspection.

Let us consider two segments, A and B, of a polypeptide chain with the same sequence of four consecutive residues. In one of them (segment A) there is a strong bond, say a covalent one, between the side chains of residues 1 and 4. No such bond exists in the other (segment B). What differences between free energies of a coil-to-helix transition can be expected for these segments? Some of them arise from side chain-backbone, side chain-solvent, and backbone-solvent interactions, because the relative orientation of the backbone and the side chains of residues 1 and 4 can be quite different in peptides A and B. It is not possible to tell in which case, in general, these interactions are more favourable for the helical conformation. What we can try to evaluate is a difference between the conformational entropy drop related with helix formation in both segments.

In segment A the bond between the side chains reduces the number of possible conformations of the backbone segment situated in between. Therefore, the helical conformation is promoted. However, this effect can be counterbalanced or even dominated by a loss of the conformational freedom of the side chains of residues 1 and 4 accompanying helix formation. In segment B the unbonded side chains can retain much of their mobility even after the helix is formed.

As we can see, there is no reason to expect that an interaction between side chains of residues i and $i \pm 3$ should stabilize, as a rule, the α-helical conformation. An attraction between residues i and $i \pm 4$ may be more effective, since it reduces the conformational entropy of a longer backbone segment. This is perhaps the reason why the statistical analysis of helical sequences in proteins shows a correlation between the occupancy of positions i and $i \pm 4$ but not of positions i and $i \pm 3$ [22].

The benzyl ring of Phe 7 in PF interacts with the side groups of many residues, even quite distant ones (e.g., the Gly 13 α-carboxyl group). Such interactions must induce some changes in a statistical distribution between various conformational forms of the polypeptide backbone. The changes are not visible in the CD spectra which are the same for PA and PF. It does not mean that they are negligibly small. CD measurements are particularly useful for detection of ordered conformations, such as helices and β-sheets, where the same pattern of backbone conformation is repeated by many peptide units. On the other hand, unordered structures, even rigid, 100% populated ones, are as a rule hardly distinguishable from the random coil conformation.

In C-peptide, Phe 8 may be expected to interact effectively with the His 12, Glu 9 and Ala 4, 5 and 6 side groups. The possible effects of these interactions on C-peptide conformation can be important and deserve careful examination.

Acknowledgements

We are deeply grateful to Professor B. Roques (Universite Rene Descartes, Paris V) and Professor J. Dabrowski (Max-Planck-Institut für Medizinische Forschung, Heidelberg) for access to NMR facilities and to Professor U. Ragnarsson (University of Uppsala Biomedical Center) for access to a peptide synthesizer. We thank Drs. M. Delepierre and A. Ejchart for their help in NMR measurements. Critical discussions and the help of Professor K.L. Wierzchowski are gratefully acknowledged. This work was supported by the Polish Academy of Sciences with Project 09.7.

References

1 a A. Bierzyński, Comments, Mol. Cell. Biophys. (1986) submitted for publication.
1 b A. Bierzyński, P.S. Kim and R.L. Baldwin, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 2470.
2 P.S. Kim, A. Bierzyński and R.L. Baldwin, J. Mol. Biol. 162 (1982) 187.
3 J.L. Nieto, M. Rico, M.A. Jimenez, J. Herranz and J. Santoro, Int. J. Biol. Macromol. 7 (1985) 66.
4 K.R. Shoemaker, P.S. Kim, D.N. Brems, S. Marqusee, E.J. York, I.M. Chaiken, J.M. Stewart and R.L. Baldwin, Proc. Natl. Acad. Sci. U.S.A. 82 (1985) 2349.
5 P.S. Kim and R.L. Baldwin, Nature 307 (1984) 329.
6 M. Rico, J.L. Nieto, J. Santoro, F.J. Barmejo, J. Herranz and E. Gallego, FEBS Lett. 162 (1983) 314.
7 M. Rico, E. Gallego, J. Santoro, F.J. Bermejo, J.L. Nieto and J. Herranz, Biochem. Biophys. Res Commun. 123 (1984) 757.
8 R.T. Ingwall, H.A. Scherega, N. Lotan, A. Berger and E. Katchalski, Biopolymers 6 (1968) 331.
9 S. Ihara, T. Ooi and S. Takahashi, Biopolymers 21 (1982) 131.
10 M.M. Hamed, R.M. Robinson and W.L. Mattice, Biopolymers 22 (1983) 1003.
11 R.B. Merrifield, J. Am. Chem. Soc. 85 (1963) 2149.
12 R.B. Merrifield, Biochemistry 3 (1964) 1385.
13 R.A. Houghten and C.H. Li, Anal. Biochem. 98 (1979) 36.
14 E. Gross and B. Witkop, J. Biol. Chem. 237 (1962) 1856.
15 A. Bundi and K. Wüthrich, Biopolymers 18 (1979) 285.
16 A. DeMarco, J. Magn. Reson. 26 (1977) 527.
17 J.L. Markley, Acc. Chem. Res. 8 (1975) 70.
18 J.E. Brown and W.A. Klee, Biochemistry 10 (1971) 470.
19 K. Rosenheck and P. Doty, Proc. Natl. Acad. Sci. U.S.A. 47 (1961) 1775.
20 S. Brahms and J. Brahms, in: Biomolecular structure, conformation, function and evolution, ed. R. Srinivasan (Pergamon Press, Oxford, 1981) vol. 2.
21 T. Tran, K. Lintner, F. Toma and S. Fermandijan, Biochim. Biophys. Acta 492 (1977) 245.
22 F.R. Maxfield and H.A. Scheraga, Macromolecules 8 (1975) 491.