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On the interactions of charged side chains with the α-helix backbone

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The effects of the position of charged amino acid side chains on the stability of the α-helix are investigated. Calculations for the model polyAla 13 residue α-helix, with modifications based on experimental work, are performed at three levels of approximation. The observed stabilization of the α-helix could be explained by interactions between its macrodipole and charged amino acid side chains. Limitations of the model are discussed.

1. Introduction

Proteins are among the most complicated structures studied in present-day science. A full understanding of the forces that determine their secondary and tertiary structures, thereby endowing proteins with their essential function, is not yet available. At present, only for small molecules of the size of several atoms is theoretical chemistry able to provide structure predictions of accuracy comparable to that of the most sophisticated experiment [1].

One approach to the analysis of complicated systems is to dissect them into smaller fragments with definite properties. Thus, peptide bonds, amino acid side chains, α-helices, β-sheets, etc., are examples of such fragments in proteins. Investigation of the properties of these isolated units can, in principle, provide us with simple, but useful models of protein properties.

However, for a long time it was believed that protein fragments could not adopt a definite conformation by themselves, but only via tertiary interactions with other parts of the whole protein. In recent years, numerous data have been obtained showing that this is not true [2]. The most referred to in this context are experiments on the α-helical conformation of isolated C-peptide (residues 1–13 of RNase A) [3]. In C-peptide, helix formation is an intramolecular process [4]. The helix is stabilized by pH-dependent interactions between side chains [4]. Two residues, Glu_2^- and His_{12}^+, contribute directly to the helix stability, as the helix-coil transition in C-peptide follows the deprotonation curve of the His_{12} residue at higher pH values while Glu_2 protonation (after correction for the destabilizing effect of Glu_9) can explain the titration curve on the acid side [3].

Apart from a few specific interactions, it has also been suggested that the interaction between the helix dipole and charged amino acid residues explains the observed increase in helix stability resulting from introduction of positive charges on the C-terminus and negative charges on the N-terminus of the helix [5]. This suggestion has been tested in an extensive series of experiments on C-peptide and a number of analogues containing various sequences of amino acids [5].

The observation that the α-helix as a whole as a strong dipole moment has led to a number of

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suggestions concerning its role in protein function and structure (for an extensive review, see ref. 6). The problem of the interactions between charged side chains and a helix dipole has been noted in the context of the statistical analysis of their distribution in α-helices [7,8] and in the postulated mechanism of helix initiation [9]: however, only recently has this problem gained more attention.

Using three levels of approximation, we discuss here the interactions of charged side chains with helix backbone for a model α-helix, with modifications based on the experimental work [5], and compare predictions of various models with experimental data.

At the first level of approximation, only the interactions between side chains and point charges located on the helix ends are calculated (level I). The second level (level II) introduces dipoles located on the peptide bonds. Finally, atom-atom electrostatic and Lennard-Jones interactions are used to calculate the total energy of the system (level III).

Three levels of approximation are introduced to evaluate the relative importance of the electrostatic energy contribution as compared to other energy terms and to ascertain the importance of the fine structure of the electrostatic field. Also, the limitations and deficiencies of each of the approximations are more clearly demonstrated by comparison with others. Moreover, when making predictions based on simple models, it is extremely important that one is aware of their limitations.

2. Details of the calculations

The molecular mechanics calculations reported in this paper (level III) were performed using the ECEPP/2 algorithm [10]. The energy was calculated with standard ECEPP energy parameters and residue geometry, as a sum of atom-atom electrostatic (charge-charge), nonbonding (Lennard-Jones) and torsional energy terms. This energy can be divided into contributions from interactions between various parts of the molecule. The value of the dielectric constant was taken as equal to 2, solvent effects not being taken into account. Energy was minimized using numerical approximations to the Hessian matrix [11]. Starting conformations for the minimization were taken from the library of sterically allowed conformations of amino acid residues in the α-helix conformation [12].

For level II calculations, dipoles of magnitude 3.5 debye have been assigned to the peptide bonds. The value and position of point charges on the helix ends (level I) have been fitted to the electric field of the peptide bond dipoles using subroutine MINUIT [13]. In both calculations the geometry and charge distribution of the charged amino acid side chains have been taken from ECEEP/2 calculations.

3. Interactions of charged amino acid side chains with the helix backbone

To determine the relative importance of the positions of charged side chains for helix stability, we performed the following numerical experiment. The energy of a poly-Ala, 13-residue-long α-helix, with one alanine substituted by either His^+ or Glu^-, was calculated as a function of the substituted amino acid position within the polypeptide chain. For each position of the substituted amino acid, all possible sterically allowed conformations of its side chain (4 for His^+, 12 for Glu^-) were investigated. We observed striking, almost linear, changes in the system's energy as His^+ or Glu^- residues were moved from the N- to the C-terminus of the helix (see fig. 1). On average, the energy change corresponding to a change in position of a charged residue is approx. -1.5 and $+1$ kcal/mol per position for His^+ and Glu^-, respectively. These effects are characteristic only for the charged forms of Glu and His, neutral forms showing no preference of the C- or N-terminus (see fig. 1).

The helix macrodipole originates from favorable alignment of the dipoles of individual peptide units and can be approximated by placing point charges of magnitude $0.5e$ and $-0.5e$ at the N- and C-terminus of the helix, respectively [6]. This approximation reproduces correctly the large-scale electrostatic potential map of the α-helix [14]. To

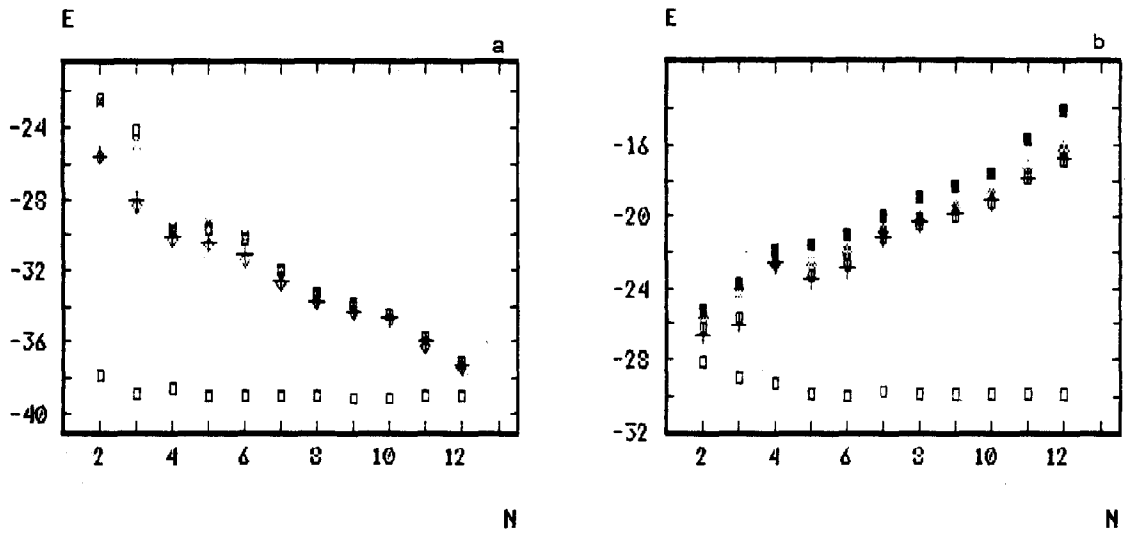

Fig. 1. Energy (in kcal/mol) of the polyAla 13-residue α-helix, with a charged side chain substituted in position *N*. (a) Conformations of His^+; (b) 5 conformations of Glu^-, the remaining conformations being omitted for clarity. Open squares in each figure show the energy of one of the uncharged conformations. All energies were calculated in the molecular mechanics approximation with the ECEPP/2 algorithm.

test the suggested role of the helix dipole, the same plot as in fig. 1 was drawn for the interaction between a charged residue and the dipoles of individual peptide units or with charges placed on the helix ends. The analogs of fig. 1 corresponding to these two situations are shown in figs. 2 and 3, in which one can observe that both approximations give similar overall energetic effects.

There are interesting differences between the three approximations. For His^+, full energy calcu-

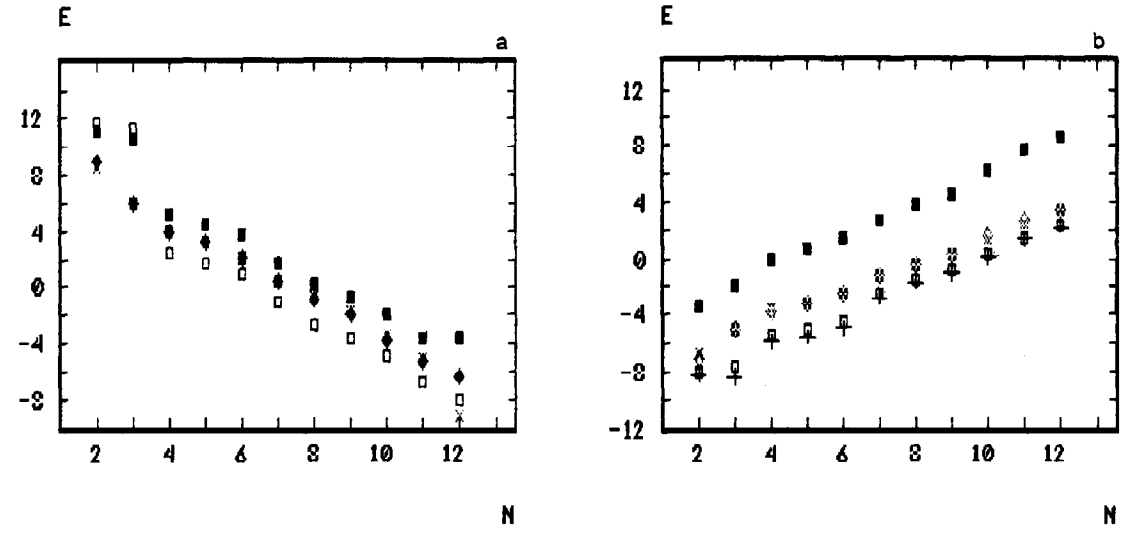

Fig. 2. Energy (in kcal/mol) of the polyAla 13-residue α-helix, with a charged side chain substituted in position *N*. (a) Conformations of His^+; (b) 5 conformations of Glu^-; the remaining conformations have been omitted for clarity. All energies calculated in the peptide bond dipole approximation (see text).

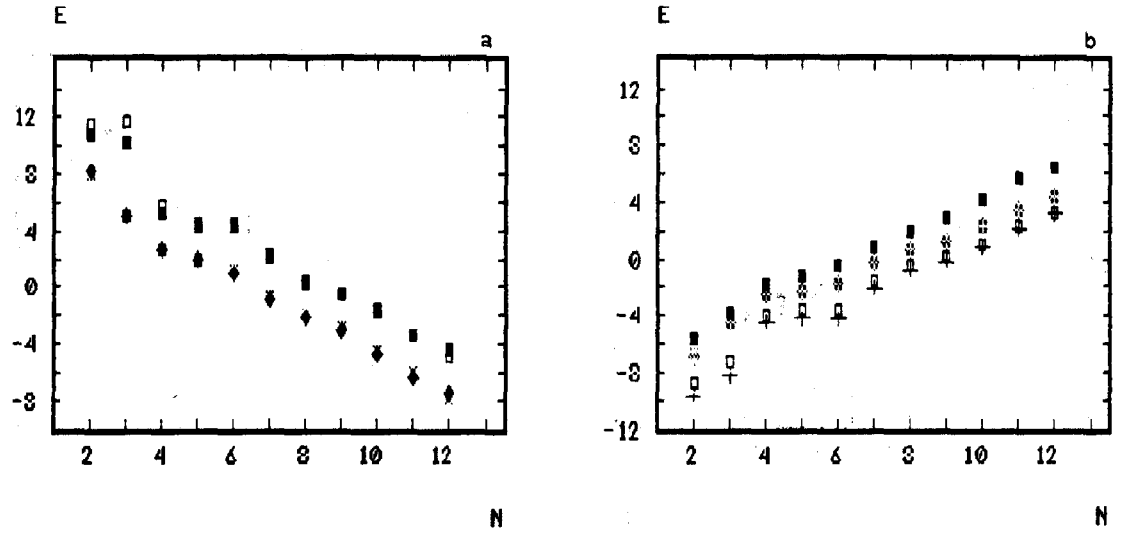

Fig. 3. Energy (in kcal/mol) of the polyAla 13-residue α-helix, with a charged side chain substituted in position N. (a) Conformations of His^+; (b) 5 conformations of Glu^-; the remaining conformations have been omitted for clarity. All energies calculated in the helix dipole approximation (see text). Symbols for different minima are consistent with those in fig. 2.

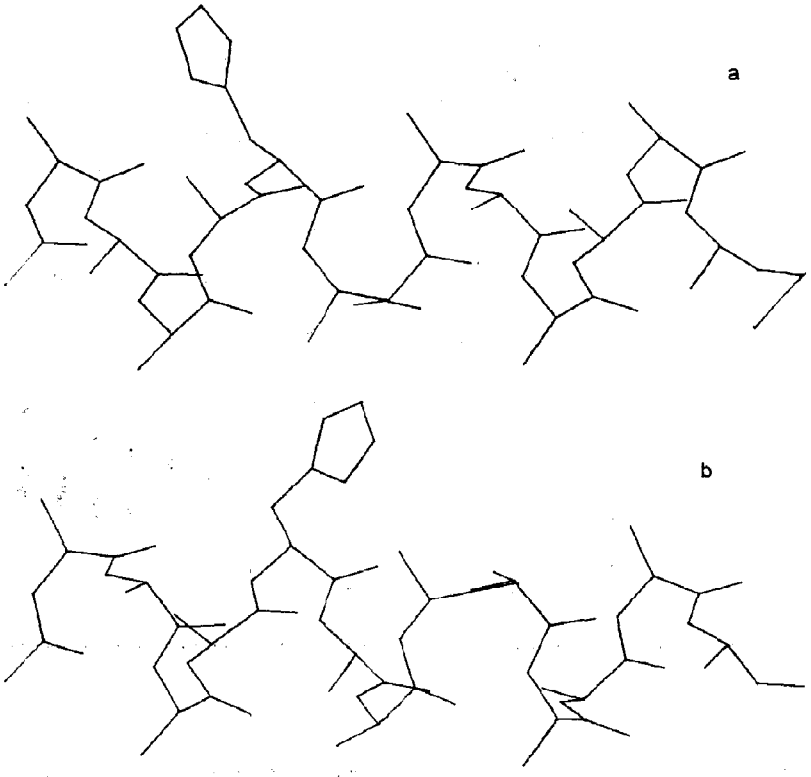

Fig. 4. Two conformations of the His^+ side chain (substituted at position 7), with different orientations with respect to the helix axis. (a) Conformation 1 (open squares in figs. 2 and 3); (b) conformation 3 × in figs. 2 and 3).

lations suggest that at the C-terminus the energy of the different conformers is the same to within 0.5 kcal/mol, on the other hand both simpler approximations predict larger (> 4 kcal/mol) energy differences and suggest that structural changes in the side chain are more important than its positional change within the helix. The conformations of His can in fact be divided into two distinct classes with different orientation of the imidazole ring with respect to the helix backbone (see fig. 4). Its relative displacement is about 3 Å and is roughly equal to a shift of two positions within the helix. Indeed, as shown in fig. 3a, the energy of minimum no. 1 at position n is almost the same as that of minimum no. 3 at position $n-2$. However, minimum no. 1, with the imidazole ring pointing in the direction of the N-terminus, and thus interacting unfavorably with charges located at the helix ends is close to the carboxyl group of the $i-4$ peptide bond unit (see fig. 5). Consequently, the electrostatic energy within the dipole approximation (level II) gives the reverse picture, i.e., minimum no. 1 now has the lowest energy (see fig. 2a). Van der Waals interactions, however, compensate for these differences and within the molecular mechanics approximation all conformers have similar energy.

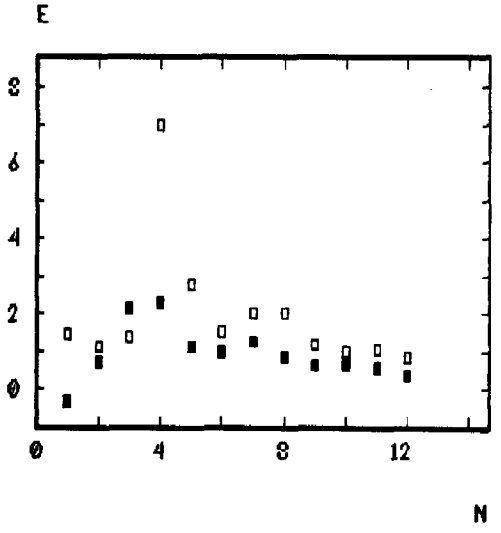

Fig. 5. The difference between $E(\mathrm{His}_i^+, \mathrm{pb}_{i+n})$ and $E(\mathrm{His}_i^+, \mathrm{pb}_{i-n})$, averaged over i, as a function of n. $E(\mathrm{His}_i^+, \mathrm{pb}_{i+(-)n})$, interaction energy of His^+ substituted at position i with the $i+(-)n$ peptide bond. Open squares, conformation 1; filled squares, conformation 3.

For the more flexible side chain of Glu^-, the differences between the various approximations are smaller.

Detailed analysis of the changes in various energy contributions suggests that the energy differences for positional change of the charged side chain are almost entirely due to electrostatic interactions between charged side chains and atoms of the peptide bonds in the α-helix backbone. Due to the high symmetry of the dipole arrangement in the α-helix, the overall effect of interactions with real atoms can be described in a simplified way as interaction with charges placed on the helix ends. This is valid only when the short-range interactions are constant, which is not always the case, for instance, the different conformers of the bulky side chains.

4. Conclusions

It has been shown that a simple dipole model for the α-helix electrostatic field is consistent with the observed stabilization of the helix by charged side chains. However, detailed considerations concerning the conformations of these side chains, as probed for instance by NMR shifts for the protons of Phe_8 and His_{12} [15], are clearly beyond its predictive possibilities.

With increasing knowledge of the details of the structure of C-peptide will arise the need for more accurate models of the interactions between its constituents. Its small size, which makes it a relatively simple subject for both experimental and theoretical investigations, also makes it an ideal testing ground for various models and hypotheses.

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References

1 W.A. Goddard, III, Science 227 (1985) 917.
2 A. Bierzynski, P.S. Kim and R.L. Baldwin, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 2470.
3 A. Bierzynski, Comments Mol. Cell. Biophys. 4 (1987) 189.
4 P.S. Kim, A. Bierzynski and R.L. Baldwin, J. Mol. Biol. 162 (1982) 187.
5 K.R. Shoemaker, P.S. Kim, E.J. York, J.M. Steward and R.L. Baldwin, Nature 326 (1987) 563.
6 W.G.J. Hol, Prog. Biophys. Mol. Biol. 45 (1985) 149.
7 P.Y. Chou and G.D. Fasman, Biochemistry 13 (1974) 211.
8 A. Wada and H. Nakamura, Nature 293 (1981) 757.
9 D.E. Blagdon and M. Goodman, Biopolymers 14 (1975) 241.
10 G. Nemethy, M.S. Pottle and H.A. Scheraga, J. Phys. Chem. 87 (1983) 1883.
11 J.E. Dennis, D.M. Gay and R.E. Welsch, ACM Trans Math. Software 7 (1981) 369.
12 L. Piela, G. Nemethy and H.A. Scheraga, Biopolymers 26 (1987) 1273.
13 F. James and M. Roos, CERN Program Library (1978) Subroutine D506, CERN Geneva.
14 R.P. Scheridan and L.C. Allen, Biophys. Chem. 11 (1980) 133.
15 M. Dadlez, A. Bierzynski, A. Godzik, M. Sobocinska and G. Kupryszewski, Biophys. Chem. 31 (1988) 175.