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THE ELECTROSTATIC POTENTIAL OF THE ALPHA HELIX (ELECTROSTATIC POTENTIAL/α-HELIX/SECONDARY STRUCTURE/HELIX DIPOLE)

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The active sites of many enzymes are very close to the N-terminus of an α-helix. The helix dipole has been postulated to enhance the binding of anions and speed charge relays in catalysis. We present electrostatic potential maps of α-helices of various lengths using a point charge model. We show that the potential field of the helix can be mimicked by two equal and opposite charges, one at each terminus. The magnitude of these equivalent charges reaches its limiting value of ± 0.2 to 0.3 electron at a helix length of approximately 7–10 residues. We also comment on the relative importance of the helix dipole to that of ionized residues in determining the electrostatics of a protein and discuss what consequences this has for enzymology.

1. Introduction

The coenzyme and substrate binding sites of many dehydrogenases, proteolytic anzyems, and other proteins are very close to the N-terminal end of α-helices. (No analogous sites have been found at the C-terminus.) It has been postulated that the large dipole of the helix [1] acts to stabilize the presence of anions at the N-terminus [2]. The dipole would enhance the binding of phosphate-containing substrates (e.g. glyceraldehyde phosphate) and coenzymes(e.g. NAD). In the case of proteolytic enzymes, backbone NH's, hydrogen bonded to the carbonyl oxygen of substrate, form an oxyanion pocket which stabilizes the tetrahedral intermediate, and in some of these (e.g. papain and subtilisin) the N-terminal end of a helix is near the pocket and would be expected to provide extra stabilization to the intermediate. The nucleophilic cysteine sulfur and serine oxygen in, respectively, papain and subtilisin are also at the N-terminus and the dipole would be expected to speed the transfer of protons in the charge relays in these enzymes. Van Duijnen et al. [3] have carried out ab initio calculations on the active site of papain under the influence of the active site helix dipole. The dipole does indeed appear likely to reduce the barrier for proton transfer.

Hol et al. [2] have discussed the electric field of the α-helix as calculated from the dipole moment of each peptide group. In this paper we discuss the electrostatic potential field around helices of various lengths. We show that the maximum potential at the ends of helices reaches a nearly limiting value at a length of about 7–10 residues. We also comment on the relative importance of the helix dipole to that of ionized residues in determining the electrostatics of a protein and discuss the consequences for enzymology.

2. Methods

We represent the α-helix with a Mulliken point charge model wherein each atom is replaced by a point charge located at its center. The magnitude (and sign) of each point charge is the net atomic charge derived from the Mulliken electron population on the corresponding atom in a model compound as calculated by molecular orbital methods. Here we use the Mulliken net charge library for amino acid residues from Hayes and Kollman [4];

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it was derived from ab initio calculations (STO-3G basis set) on small residue models. We and others [5,6] have demonstrated that for small molecule analogs of the residue backbone and sidechains the electrostatic potential from the point charge model qualitatively mimics the potential as calculated from the molecular wavefunction. The dipole moment of the point charge model for peptide groups is underestimated relative to the moment from the wavefunction by a factor of about 1.3. The magnitude of dipole moments calculated from wavefunctions are also basis set dependent. Thus the dipole moment computed from STO-3G wavefunctions are underestimated relative to the experimental moment by an additional factor of 1.2. In total, the electrostatic contribution from the peptide group dipoles in the point charge helix will be underestimated by a combined factor of 1.6 relative to a monopole charge.

Investigations on the electrostatic potentials calculated from molecular wavefunctions show that polarization in a molecule induced by hydrogen bonding and/or other perturbations does not significantly affect the potential. That is, the potential of two interacting peptide units is essentially the sum of the potentials of each unit. Cooperativity in interpeptide hydrogen bonds [7] will also not affect the potential significantly. Thus the point charge model derived for isolated residues should be adequate for the study of the helix potentials.

All helices are constructed in units of the residue NHCHRCO. The fact that the terminal N and C of the helix backbone are "uncapped" by atoms introduces a very small artifact which becomes insignificant for helices longer than about five residues.

The position of the atoms in the helix is taken from the crystallographic study of polyalanine by Arnott [8]. All electrostatic potential maps were produced from a modified version of the program PTCHG, developed in our laboratory by Fisher [9].

3. Results and discussion

Electrostatic potential maps for the polyalanine α-helix of 1, 5 and 15 residues appear in fig. 1. It should be pointed out that in globular proteins the helices are not necessarily as regular as in polyalanine, i.e. the orientation of the moments of the peptide groups relative to the helix axis may vary somewhat. This will slightly reduce the total moment along the axis, but otherwise the electrostatic features of the polyalanine

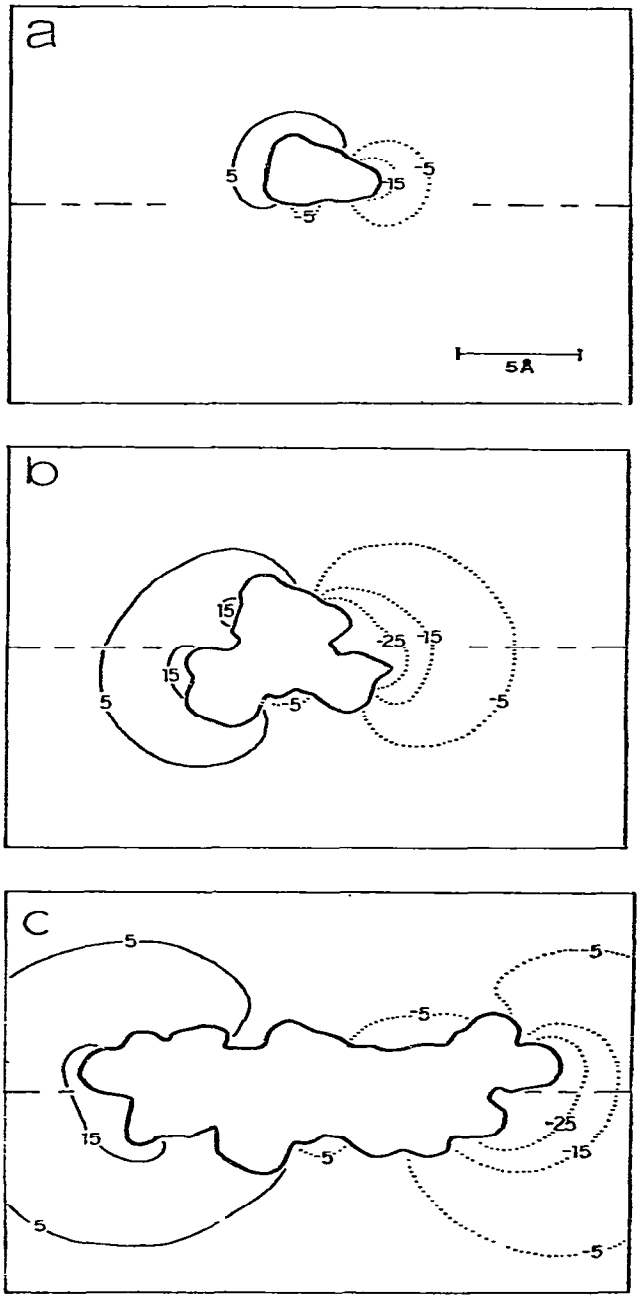

Fig. 1. Electrostatic potential maps of α-helices of various lengths. Plane of cross section through helix axis (broken line) passing through the C_α of the middle residue. The C-terminus is to the right. Contour levels are in kcal. a. (Ala), b. (Ala_5), c. (Ala_{15}).

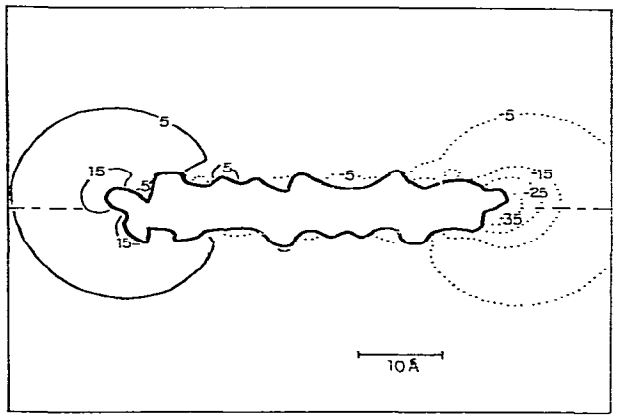

Fig. 2. A very long helix. (Ala_{31}).

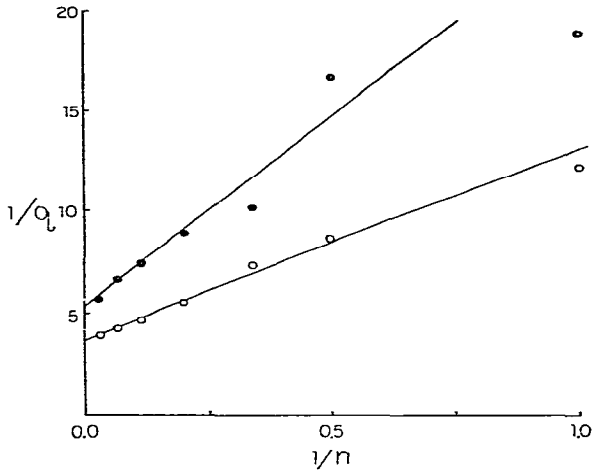

Fig. 3. Double-reciprocal plot: $1/q$ versus $1/n$. q is the magnitude of the effective charge at the helix termini. n is the number of residues. Closed circles, q estimated from the radius of a contour line; open circles, q estimated from distance between centroids of charge and dipole moment. The deviations from linearity as $1/n$ approaches 1 stem from the difficulty of making reliable estimates of radius or distance for small helices.

helix should carry over to helices in proteins.

The apparent centroid of charge at the C-terminus seems to be one or two Ångström outside the van der Waals envelope of the helix. This is reflected by the greater number of contour lines near the C-terminus. This effect is not dependent on which cross section of the helix is taken. In contrast, the centroid for the N-terminus is always within the van der Waals envelope. At larger distances from the envelope the positive and negative contours are very nearly symmetric about the midpoint of the helix. The potential map of a helix appears equivalent to that produced by two equal and opposite point charges, one at each end of the helix. This is most obvious in very long helices. See fig. 2. The effective magnitude of the equivalent charges may be estimated in a number of ways, as discussed below.

The nearly circular shape of the contour lines indicates that (except for points very close to the van der Waals envelope) the potential depends only on the distance from the termini and not on angle; the axis of the helix does not have to point directly at ion binding sites in proteins for the helix potential to be effective.

Study of helices of various intermediate and very long length indicate that the radius of a given potential contour around a helix terminus approaches its maximum at a length of 7–10 residues. Compare figs. 1 and 2. Hol et al. [2] take 7 residues as the length producing near maximum electric field strength as calculated from line dipoles. It should be noted that the helices in the enzymes cited by Hol et al. are from 7 to 19 residues long. Thus, all have essentially the same maximum potential at the N-terminus. The change in radius of a given potential contour with length can be equally well represented by the change in magnitude, q, of the equivalent point charges at the ends of the helix. We give two ways of estimating q. First, we can measure the radius of a nearly circular contour line (the radius for the N- and C-termini are essentially the same) and calculate the charge necessary to produce a contour of that radius in the plane of cross section. As a second method we measure the distance between the apparent centroids of charge at the termini and divide this into the point charge model dipole along the direction of the helix axis. The limiting value of q is obtained from a double-reciprocal plot extrapolated to $1/n \rightarrow 0$ (infinitely long helix). Fig. 3 yields a limiting q of ± 0.2 to 0.3 electron charge. Since, as noted before, the point charge model underestimates the dipole moment of the peptide and ignores the very small contribution of polarization in hydrogen bonds, the limiting q should be scaled up by a factor of slightly over 1.6. Our estimate with scaling approaches that from Hol et al. [2] of ± 0.5 electron charge.

Fig. 4a is a map for a single aspartate residue. Its potential resembles that of a unit negative charge centered at the carboxyl carbon C^{γ} of the sidechain. Figs. 4b and 4c show a 15 residue helix (of essentially maximum helix

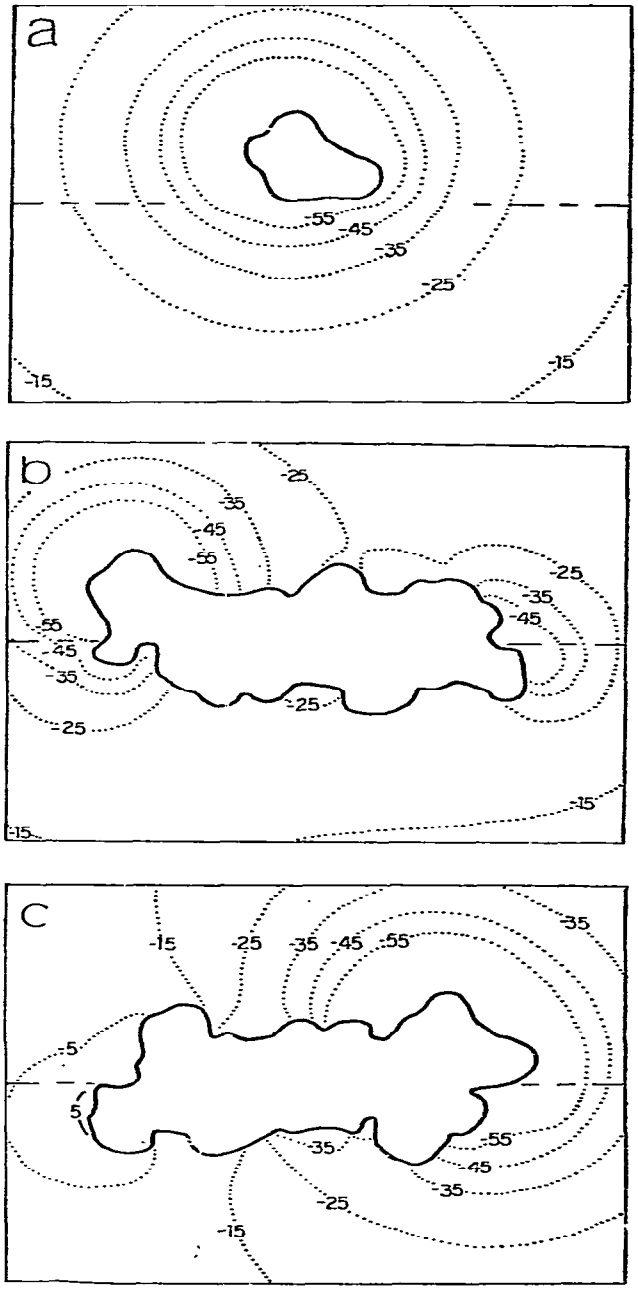

Fig. 4. Helices with an ionized aspartate residue. The plane of cross section passes through C^{γ} of Asp. a. $(Asp)^{-}$, b. $(Ala-Asp-Ala_{13})^{-}$, c. $(Ala_{13}-Asp-Ala)^{-}$.

dipole potential) with aspartate as the second and penultimate residue, respectively. The atoms of the helix partly screen the potential of the aspartate, but as can be seen, the charged residue dominates the dipole potential of the helix. This would be true even if the helix dipole potential were scaled up as noted. Hydration, the presence of counterions, and solvent dielectric will no doubt reduce the influence of both charged residues and the helix dipole, the former probably to a greater extent. Still, in order to have the helix dipole potential as the major electrostatic influence on a substrate or coenzyme binding site, a protein must sequester these sites within crevices shielded from solvent and at the same time keep any charged residues at a reasonably large distance from the site. (The exact value of this distance will depend on how much the potential of the charged residue will be reduced relative to the helix dipole potential by hydration.) If these conditions are met, anions will find stability at the N-termini of helices. On the other hand, at points somewhat removed from binding site crevices, the greater electrostatic influence is very likely to be the charged residues on the protein surface. That is, helices will stabilize the presence of anions already in the binding site, but will not draw them in from a distance.

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