Delineation of Electrostatic- and Hydrophobic-Induced pK_a Shifts in Polypentapeptides: The Glutamic Acid Residue

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Polypentapeptides of the family poly[f_V(IPGVG),f_E(IPGEG)],[1] where f_V and f_E are mole fractions with $f_V + f_E = 1$, were synthesized and characterized as to pK_a in water and in 0.15 N NaCl with f_E varied from 1 to 0.06. As the value of f_E is decreased from 1.0 to 0.7, in saline there is a slight increase in pK_a from 4.35 to 4.40 and in water there is a decrease in pK_a from 4.70 to 4.35 as the charge–charge repulsion is relieved due to dilution of charge. This is the charge–charge repulsion (electrostatic) domain. As the value of f_E is further decreased from 0.7 toward 0 and the hydrophobicity increases as Val replaces Glu, in both saline and water the pK_a increases from the value near 4.4 to a value of 6.6 in water and to a lower value of 6.1 in saline. The values of $f_E < 0.7$ represent the hydrophobic domain in which the hydrophobic-induced pK_a shift dominates. These results unambiguously delineate the domains for hydrophobic- and electrostatic-induced pK_a shifts in these polypentapeptides, and they suggest that in aqueous systems the latter is the dominant means of shifting pK_a values. Nonetheless, for values of f_E less than 0.35 in the hydrophobic domain, salt decreases the pK_a shift. Because of this, as discussed below, the decrease in affinity between protein subunits on addition of salt cannot be considered diagnostic of the electrostatic domain.

Ten polypentapeptides, poly[f_V(IPGVG),f_E(IPGEG)], with values of f_E varying from 1 to 0.06, were synthesized as previously described.[2,3] The syntheses and compositions were verified by amino acid analyses and carbon-13 nuclear magnetic resonance. The amino acid titration experiments were carried out at 37 °C, as previously described,[3] using 45 min per data point (requiring approximately 24 h for each of three or more titrations per reported pK_a value) and both in Type 1 (low-conductivity) water and in 0.15 N NaCl. The plots of f_E versus pK_a are given in Figure 1 for water and saline.

The plots in Figure 1 readily divide into two domains with respect to f_E: $f_E > 0.65$ and $f_E < 0.65$. In water, the lowering of the pK_a on going from an f of 1.0 to 0.7 is due to the removal of the charge–charge repulsion-induced pK_a shift as the negatively charged carboxylate side chains of Glu become more dilute. In salt, 0.15 N NaCl, the charge–charge repulsion is shielded by the ions in solution. The $f_E > 0.65$ values are clearly within the electrostatic domain. For the range of values $f_E < 0.65$ in both water and physiological saline, as the mole fraction of Glu-containing pentamers decreases below 0.65, the increases in pK_a become progressively greater as the mean hydrophobicity increases. This is the hydrophobic, or apolar–polar repulsive interaction, domain. It is important to note that even in the hydrophobic domain, for values of f_E of 0.35 and less, the effect of salt to reduce the pK_a shift is the same as in the electrostatic domain. This is discussed in terms of the competition of apolar and polar moieties for hydration in a structurally-constrained, and hence, water-limited, polymer system.

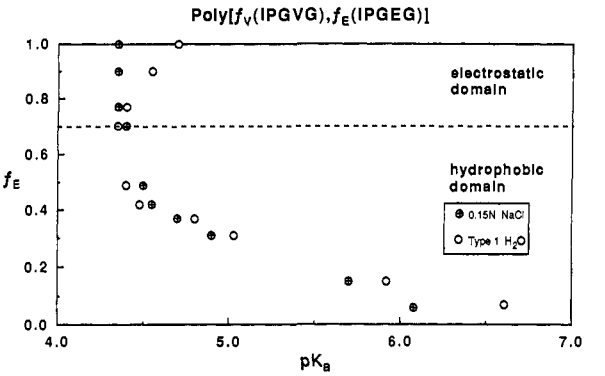

Figure 1. pK_a values of glutamic acid determined using classical acid–base titrations for poly[f_V(IPGVG),f_E(IPGEG)], where f_V and f_E are mole fractions of pentamers and $f_V + f_E = 1$, with values of f_E ranging from 1 to 0.06 in water and in 0.15 N NaCl at 37 °C. Each value is the mean of three or more titrations, with standard derivatives ranging from 0.01 for high values of f_E to 0.11 for the lowest value of f_E.

The broader family of polypentapeptides, poly[f_V(VPGVG)$f_{\alpha\beta}$-(αPGβG)], where f_V and $f_{\alpha\beta}$ are mole fractions with $f_V + f_{\alpha\beta} = 1$, where α may be one of a few hydrophobic residues, e.g., Val, Ile, Phe, etc., and where β may be any of the naturally occurring amino acid residues and chemical modifications thereof, exhibits an inverse temperature transition in which all members are soluble in water, if low enough temperatures can be reached, and on raising the temperature they hydrophobically fold and assemble in a phase separation as the more-structured water of hydrophobic hydration becomes less-structured bulk water. The temperature T_t at which the inverse temperature transition occurs depends on the hydrophobicity of the α and β residues, and a complete T_t-based hydrophobicity scale has been obtained for all of the naturally occurring amino acids and certain chemical modifications thereof.[4]

Increasing the degree of ionization of a Glu side chain strikingly increases the value of T_t.[5] Also from differential scanning calorimetry, the endothermic heat of the transition, interpreted as the heat required to destructure the hydrophobic hydration, is seen to decrease as the degree of ionization increases.[6] This, along with other data,[5] indicates that the polar species, in the process of achieving their hydration shells, destructure the water of hydrophobic hydration. Conversely, as the hydrophobicity increases, it becomes more difficult for an emerging carboxylate anion to achieve its required hydration shell, as this necessitates destructuring of hydrophobic hydration. The consequence is the hydrophobic-induced pK_a shift.[3,7] The free energy for this competition for hydration, called an apolar–polar repulsive free energy of hydration, can be obtained from the pK_a shift, i.e., $\Delta\mu = -2.3RT\delta pK_a$ where $\Delta\mu$ is the change in chemical potential of the proton indicated by the pK_a shift, R is 1.987 cal/mol deg, and T is in Kelvins. With the largest hydrophobic-induced pK_a shift being from 4.35 to 6.61, the $\Delta\mu$ (the Gibbs free energy per mole) arising from the apolar–polar repulsive free energy of hydration is 3.2 kcal/mol.

Waters of hydrophobic hydration, in general, cannot simultaneously serve as waters of polar hydration.[5] Stated macroscopically, this means a lower effective dielectric constant surrounding the negatively charged moieties. Similar pK_a shifts

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(1) The single letter codes for the amino acid residues are Gly(G), Val(V), Ile(I), Pro(P), and Glu(E).

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are seen for positively charged species, where increased hydrophobicity results in a decrease in the pK_a of a lysyl side-chain ammonium moiety (D. W. Urry, S. Peng, D. C. Gowda, T. Parker, and R. D. Harris, unpublished results). Again, the effective dielectric constant surrounding the ammonium moiety could be considered to decrease with increased hydrophobicity. Therefore, if two protein subunits had interfacial lysine and glutamic acid residues with hydrophobically shifted pK_a values, the Coulombic attraction between the two would be increased. That attraction, due to the hydrophobic domains in which the Lys and Glu residues were located, would be decreased by the addition of salts. Analogously, the addition of salt to the medium of a protein with a hydrophobically shifted pK_a of a Glu residue would, by providing counterions, relax the apolar–polar repulsive free energy of hydration and decrease the magnitude of the pK_a shift, as seen in Figure 1 for f_E for less than 0.35.

In Figure 1 the electrostatic-induced pK_a shift is less than the hydrophobic-induced pK_a shift. The charge density can, of course, be increased in polypeptides or proteins to more than occurs in these polypentapeptides, that is, to more than one carboxylate per five residues which is a charge density of one carboxylate per 15 backbone atoms. The charge density could not be made as great as in poly(methacrylic acid) where there can be one carboxylate for every two backbone atoms. With the very large charge–charge repulsion in poly(methacrylic acid), the pK_a is raised only to 7.3.[8] On the other hand, the hydrophobicity can also be greatly increased. With an f_E of 0.17 and with five Phe residues per tricosamer, a pK_a of 8.1 has been observed.[9] Even larger shifts could conceivably occur for Glu residues in globular proteins with sufficiently hydrophobic domains or in membrane proteins. Thus it is not unreasonable to expect that the larger pK_a shifts are due dominantly to the apolar–polar repulsive free energy of hydration.

Amino acid residues with shifted pK_a values have been increasingly identified as key to protein function whether observed in globular proteins in solution[10–15] or in proteins in membranes.[16,17] Accordingly, as the apolar–polar repulsive free energy of hydration is the most effective means of shifting pK_a values, it should be considered to be significant in general in protein mechanisms. More specifically, the apolar–polar repulsive free energies of hydration have been used to achieve free energy transductions involving the intensive variables of mechanical force, temperature, pressure, chemical potential, electrochemical potential, and electromagnetic radiation.[5,18] Furthermore, chemomechanical transduction has been demonstrated, when using the apolar–polar repulsive free energy of hydration mechanism, to be 1 order of magnitude more efficient than when using the charge–charge repulsion mechanism.[5,18]

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