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Salt Bridge Interactions: Stability of the Ionic and Neutral Complexes in the Gas Phase, in Solution, and in Proteins

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ABSTRACT **A theoretical study on the stability of the salt bridges in the gas phase, in solution, and in the interior of proteins is presented. The study is mainly focused on the interaction between acetate and methylguanidinium ions, which were used as model compounds for the salt bridge between Asp (Glu) and Arg. Two different solvents (water and chloroform) were used to analyze the effect of varying the dielectric constant of the surrounding media on the salt bridge interaction. Calculations in protein environments were performed by using a set of selected protein crystal structures. In all cases attention was paid to the difference in stability between the ion pair and neutral hydrogen-bonded forms. Comparison of the results determined in the gas phase and in solution allows us to stress the large influence of the environment on the binding process, as well as on the relative stability between the ionic and neutral complexes. The high anisotropy of proteins and the local microenvironment in the interior of proteins make a decisive contribution in modulating the energetics of the salt bridge. In general, the formation of salt bridges in proteins is not particularly favored, with the ion pair structure being preferred over the interaction between neutral species. Proteins 32:67–79, 1998.**

Key words: salt bridge; solvation; continuum models; electrostatic interactions; protein stability; proton transfer

INTRODUCTION

Salt bridges are among the more thoroughly investigated interactions in protein chemistry. This interest is due to the important and often quite specific functions played by ion pairs.[1] They act as binding sites in enzymes,[2–4] mediate molecular recognition,[5–7] and modulate the allosteric behavior[8,9] of proteins. Their role in determining the stability of secondary-structural elements has also been considered.[10] Indeed, several studies on model peptides have shown that the character and position of salt-bridging groups affect helix formation.[11–14] Salt bridges are also involved in connecting different subunits of proteins[15–19] or in the control of the equilibrium between various conformational states.[20] The importance of salt bridge cooperativity effects in joining different structural domains and in determining the mechanisms of allosteric regulation has also been explored.[21,22] Finally, salt bridges might also determine the early condensation of disordered polypeptide chains.[23]

Understanding of the structural and functional roles played by salt bridges cannot be achieved without knowledge of the energetics of this interaction, which is important for determining the contribution of salt bridges to protein stability. The finding that thermophilic proteins contain more salt bridges than their mesophilic counterparts gives support to the contribution of ion pair interactions to thermostability.[24,25] However, whether or not salt bridges make a significant stabilizing contribution is still under discussion. In general, solvent-exposed salt bridges seem to play little role in stabilizing proteins, whereas the contribution due to salt bridges that are completely or partially buried appears to be more important.[26–29] Indeed, the stabilizing contribution seems to vary sensibly between different proteins and appears to be highly dependent on factors such as the screening of charges by solvent, the cost of desolvating the charged groups to form the salt bridge, and the flexibility of the sidechains in the ion pair. At this point, Warshel et al.[30] noted that ionized groups cannot exist inside nonpolar regions of proteins. Recently, the finding that buried salt bridges can be destabilizing relative to hydrophobic residues has also been noted by several authors.[31–34] These findings, which could be relevant for protein engineer-

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ing and binding affinity, raise the question about the specific role played by buried salt bridges.

Another interesting point concerning the stabilizing contribution of salt bridges is the state of protonation of ionizable groups. A comparison of the free energies of transfer of ionized amino acid sidechains in water to both their ion-paired and neutral hydrogen-bonded states in low dielectric media suggests that the proton transfer energy is very small, which in turn suggests a similar population of both complexes.[35] However, it was also noted that the ion pair will be favored in real proteins due to the formation of additional interactions (e.g., hydrogen bonding). This suggestion agrees with the conclusions reached by Warshel et al. indicating that in nonpolar media the ion pairs are less stable than their neutral counterparts, and that the ionized groups in the interior of proteins are stabilized by the polar environment exerted by the surrounding protein permanent dipoles.[30,36,37] Recently, results derived from quantum mechanical calculations for the interaction between formate–trimethylammonium and formate–guanidinium ion pairs favor the existence of the neutral hydrogen-bond forms in low polar solvents.[38,39]

Here we present a theoretical study on the stability of the salt bridges in different media. The study is focused on the interaction between acetate and methylguanidinium ions, which were used as model compounds for the salt bridge between Asp (Glu) and Arg. Calculations were performed in the gas phase, in solution, and in the interior of proteins. Two different solvents (water and chloroform) were used to analyze the effect of varying the dielectric constant of the surrounding media on the salt bridge interaction. Calculations in protein environments were performed by using a set of selected protein crystal structures. In all cases attention was paid to the difference in stability between the ion pair and neutral hydrogen-bonded forms.

METHODS

Selection of Model System

The interaction between the ionizable sidechains of Asp (Glu) and Arg was modeled by the acetate and methylguanidinium moieties. Since the strength of the salt bridges depends on the relative geometry of the interacting fragments, it would be necessary to perform a detailed exploration of the configurational space. For this purpose, we considered as starting geometries the set of structures determined by Mitchell et al.[40] They include two "symmetric" structures (A, B in Fig. 1), with two linear N–H · · · O–C hydrogen bonds between the carboxyl oxygens and the nitrogens Nϵ and Nη1 in the "side-on" (A) interaction, and Nη1 and Nη2 in the "end-on" (B) contact. There are also two "staggered" structures (C, D), in which the hydrogen bonds deviate from linearity. Finally, the set includes two "twisted" structures (E, F), where the carboxyl group lies in a plane perpendicular to the methylguanidinium ion. Calculations were also performed for the corresponding acetic acid–methylguanidine complexes, which were generated upon transferring the hydrogen-bond proton (denoted *1* and *2* in Fig. 1) to either of the two carboxyl oxygens. Therefore, every ion pair complex was related to two neutral hydrogen-bonded forms. Finally, calculations were also performed for the doubly hydrogen-bonded complex between acetate and methylammonium ions (structure G in Fig. 1), as well as its neutral complex, for comparison purposes.

Gas-Phase Calculations

Equilibrium geometries for the ionic and neutral complexes of methylguanidinium and acetate ions were fully optimized at the Self-Consistent Field-Restricted Hartree-Fock (SCF-RHF) level using the 6-31G(d)[41] basis set. The geometry optimization did not converge to true minima of the gas-phase energy surface for some of the complexes. In these cases, the relaxed geometry was obtained from partial optimization by imposing the restraints necessary to keep the relative orientation of the monomers in the complex. Single-point calculations at the second order Møller-Plesset (MP2) level using the 6-31 + G(d)[42] basis set were performed using the HF/6-31G(d) optimized geometries. The basis set superposition error (BSSE) was corrected following Boys and Bernardi.[43]

When possible, force constant analyses were performed to verify the minimum-energy state of the final geometries. In particular, the side-on and end-on symmetric (ionic and neutral) complexes were found to be stationary points. The energy obtained at the MP2 level was corrected by the addition of thermal and entropic (298 K) effects determined at the HF/6-31G(d) level using the standard procedures in Gaussian-94[44] in order to obtain the Gibbs free energy. In cases where constraints were imposed during the geometry optimization, these corrections were taken from the values determined for the most similar true minimum-energy ionic or neutral complexes. It is expected that the uncertainties introduced from this procedure will be small.

Solvation Calculations

Self-consistent reaction field (SCRF) calculations were performed to incorporate the solvent effect on the stability of ionic and neutral complexes. In order to examine the influence of the permittivity of the medium on the stability of ionic and neutral complexes, calculations were performed in water and chloroform. The ab initio 6-31G(d) version[45–47] of the polarizable continuum model[48] formulated by Miertus, Scrocco, and Tomasi (MST) was used to determine the electrostatic component of the free energy

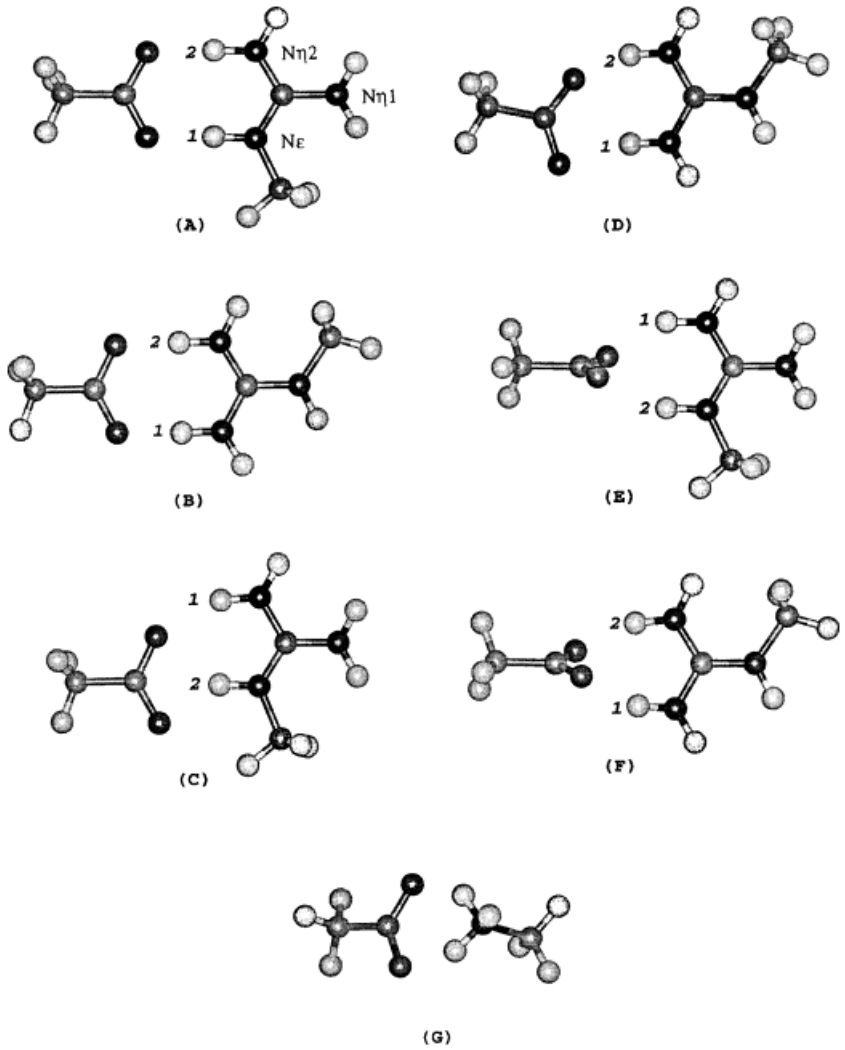


Fig. 1. Intermolecular geometries for the interaction between acetate and methylguanidinium ions. The structures correspond to side-on and end-on symmetric (**A, B**), staggered (**C, D**), and twisted (**E, F**) complexes. The geometry for the interaction between acetate and methylammonium is denoted by structure G.

of solvation.[49] The steric contribution was introduced following Pierotti's scaled particle theory,[50] and the van der Waals component was calculated by using an optimized linear relationship with the atomic surface area. The solute cavity was built up using the standard procedure,[49] but it was reduced by a factor of 0.92 for calculations involving charged species.[51]

Calculations in Proteins

The three-dimensional (3D) structures of a number of relatively small proteins containing Arg–Asp(Glu) interactions were used to examine the effect of the protein environment on the salt bridge. In all cases, the resolution of the structures taken from the Brookhaven Protein Data Bank[52] was better than 1.8 Å. The set of selected proteins was 1cse (resolutions: 1.2 Å), 1hny (1.8), 1pda (1.8), 1rro (1.3), 1try (1.6), 2end (1.5), 2pck (1.5), and 5p21 (1.4).

The electrostatic contribution to the complex formation in proteins (ΔG_{tot}) was calculated as the work necessary for bringing the interacting monomers infinitely separated in aqueous solution to their position in the protein. This work was computed as the addition of three terms (see Fig. 2): the energy required to desolvate the monomers (ΔG_{des}), the interaction between monomers in their position as a dimer in the protein (ΔG_{dim}), and the interaction with the rest of residual charges of the atoms in the protein (ΔG_{prot}). These contributions were determined by solving numerically the finite difference linearized Poisson-Boltzmann equation.[53,54]

The ΔG_{des} term was determined from the difference between the total energies of the monomers in

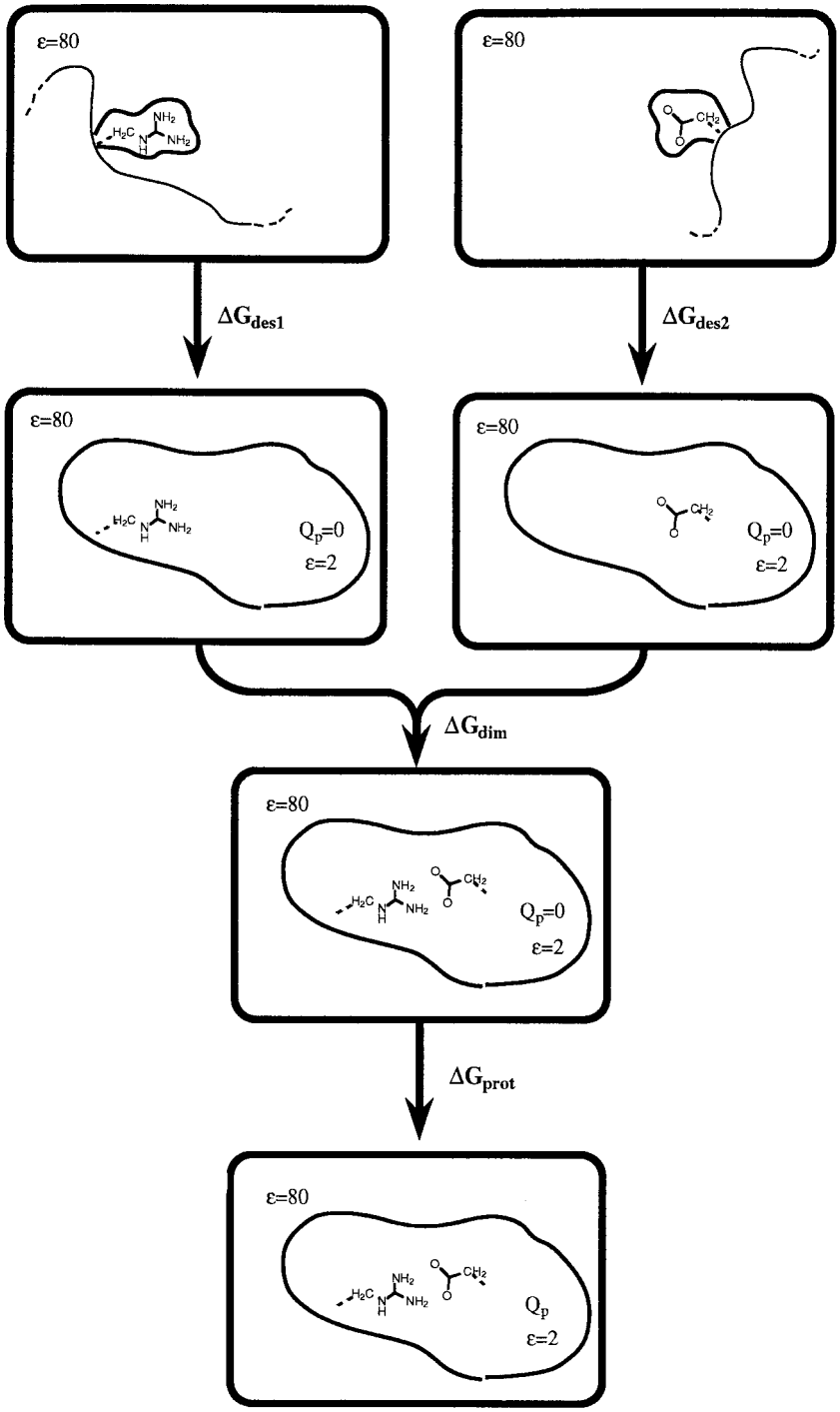


Fig. 2. Schematic representation of the three contributions to the total interaction energy of ionic and neutral complexes in the interior of the protein: desolvation of the monomers (ΔG_{des}), coulombic interactions of the monomers in the protein (ΔG_{dim}), and interaction with the rest of residues in the protein (ΔG_{prot}). The desolvation electrostatic term (ΔG_{des}) corresponds to addition of the ΔG_{des1} and ΔG_{des2}. Q_P denotes the residual charges of the protein atoms, which are activated in the last step of the diagram.

the folded and unfolded protein. In these calculations, the partial charge of all the atoms but those corresponding to each of the monomers was set to 0. The folded state was assumed to be the X-ray crystal structure, whereas the unfolded state was represented by extracting the respective sidechains from the protein structure and putting them infinitely separated in solution keeping the same coordinates. The ΔG_{dim} contribution was determined by solving the Poisson-Boltzmann equation, having assigned

partial atomic charges to the atoms in the dimer, but setting the partial charge on any other atom of the protein equal to 0. Finally, the ΔG_{prot} term was determined by solving the Poisson-Boltzmann equation with actual charges in all the atoms but those forming the dimer, and summing the products between the electrostatic potential at each atomic position in the dimer and the partial charge of that atom. Let us note that the contribution due to entropy changes is not taken into account, since it is assumed that the local loss of entropy arising upon formation of the complex is similar in all cases.

The partial atomic charges were taken from the AMBER force field,[55] except in the case of methylguanidine and acetic acid, for which electrostatic potential-derived atomic charges were computed at the HF/6-31G(d) level. Default atomic radii were used in all calculations. The dielectric constant of the solvent was set to 78.4, and that in the interior of the protein was set to 2. In order to determine the dependence of the results on the dielectric response of the protein, additional calculations were also performed using a dielectric constant of 4 and 8 for the interior of the protein. In all cases, the linearized Poisson-Boltzmann was solved iteratively using the focusing approach. At the end an average grid spacing of ~0.3 Å/grid unit was reached.

Computational Details

Gas-phase calculations were performed with Gaussian-94. SCRF calculations were carried out with a modified version of MonsterGauss.[56] Continuum electrostatic calculations in proteins were performed with the version 3.0 of the DELPHI program as implemented in Insight-II.[57] Calculations were performed on the IBM-SP2 of the Centre de Supercomputació de Catalunya, and on HP and SGI workstations in our laboratory.

RESULTS AND DISCUSSION

Gas-Phase Calculations

Table I reports the gas-phase binding energies at the MP2/6-31 + G(d) level for ionic and neutral complexes. The values were determined with regard to the minimum-energy structures for the ionic and neutral isolated monomers. The most stable form of methylguanidine was used as reference for calculating the binding energies of neutral complexes. In all cases, the binding energies were corrected for BSSE and energy changes due to geometry distortion upon formation of the complex. These energy corrections were rather uniform for the different complexes (around 4 and 3 kcal/mol for ionic and neutral complexes, respectively), and do not affect the relative stability between structures.

The binding energy of ionic complexes ranges between −118 and −108 kcal/mol. The symmetric structures are the most stable complexes, with the side-on (structure A) interaction being preferred by around 1 kcal/mol with regard to the end-on (B) complex. The loss of linearity in hydrogen bonds for staggered (C, D) structures leads to a destabilization of 4–6 kcal/mol with regard to the side-on interaction. This effect is larger (8–9 kcal/mol) for the twisted (E, F) structures, which cannot establish proper hydrogen bond interactions. Therefore, it is clear that the contribution of hydrogen-bonded interactions to the stability of salt bridges is far from being negligible. The binding energy for the acetate–methylammonium (G) complex is at least 6.0 kcal/mol more favored than the interaction between acetate and methylguanidinium, due to the larger concentration of positive charge in the methylammonium group.

TABLE I. Binding Energies[a] (kcal/mol) for Ionic and Neutral Complexes in the Gas Phase, and Energy Difference (E_{PT}) Between Hydrogen-Bonded Ionic and Neutral Complexes

Structure[b]	Ionic	Neutral[c]	E_{PT}[d]
	Methylguanidinium–acetate		
A	−117.9 (0.0)	−14.5 (0.0)	−1.7
B	−116.5 (1.4)	−14.5 (0.0)	−3.0
C	−113.7 (4.2)	−13.0 (1.5)	−4.4
D	−111.7 (6.2)	−14.3 (0.2)	−8.3
E	−110.0 (7.9)	−9.5 (5.0)	−4.0
F	−108.8 (9.1)	−9.2 (5.3)	−4.5
	Methylammonium–acetate		
G	−125.2 (−6.3)	−10.5 (4.0)	−9.8

[a]Values are determined at the MP2/6-31+G(d) level. The results were corrected for BSSE and changes in energy due to geometry distortion upon formation of the complex. Relative energies are given in parentheses.
[b]See Figure 1.
[c]Binding energies are computed relative to the most stable form of methylguanidine. Only the binding energy of the most stable neutral complex arising upon transfer of hydrogen atoms 1 and 2 from methylguanidinium (methylammonium) to acetate is given.
[d]Energy of proton transfer (PT) to form neutral complex from salt bridge.

The binding energy of neutral hydrogen-bonded complexes varies from −9.0 to −15.0 kcal/mol. The difference in stability of both symmetric (A, B) and staggered (C, D) complexes is very small (around 1 kcal/mol), but twisted (E, F) structures are disfavored by around 5 kcal/mol from the most stable complexes. The lower energy difference between symmetric and twisted forms in the neutral complexes with regard to the ionic structures reflects the larger distance (around 0.3–0.6 Å) between monomers in the former dimers. The binding energy for methylamine lies in the range of interaction energies found for methylguanidine.

Several theoretical studies focused on the formation of ionic and neutral complexes, generally involving formate (formic acid), have been reported.[58–62] The binding energies at the Hartree-Fock (HF) and

MP2 levels for the ion pair agrees well with present results. With regard to the neutral complexes, the results for structures A and G lies in the range of values determined for the doubly hydrogen-bonded dimer of formic acid, whose interaction energy varies from −11 to −15 kcal/mol using very high levels of theory (up to MP4, QCISD(T) and G2).[63] It also compares with the experimental dimerization enthalpy of the formic acid (around −14.5 kcal/mol).[63]

Table I also reports the energy of proton transfer to form the hydrogen-bonded neutral complex from the ionic (salt bridge) structure. The results clearly indicate that the neutral complexes are energetically more favored than the ionic structures in all the cases. This trend is also found for the interaction between methylamine and acetic acid, even though the energy difference is sensibly larger than for the methylguanidinium–acetate complex. Our results agree with previous theoretical values of the energy change arising upon proton transfer in the ionic complex. Thus, such an energy difference was estimated to be around −4 kcal/mol for the guanidinium–formate complex.[38] It is also satisfactory that our estimate for the proton transfer in methylammonium–acetate (−9.8 kcal/mol) lies between the values reported for formate–ammonium (around −11 kcal/mol) and formate–trimethylammonium (near −6 kcal/mol).[39,64]

Table II shows the free energy differences for the binding of ionic and neutral monomers. The change in zero-point energy and thermal corrections upon binding amounts, in general, to 2 kcal/mol. The most important contribution arises from the entropy term, which leads to a destabilization of around 10 kcal/mol. As a result, the binding free energy between formate and methylguanidinium varies from −106 to −96 kcal/mol, and amounts to −113 kcal/mol for formate–methylammonium. In the case of neutral complexes, the binding is favored for the symmetric (A, B) and staggered (C, D) structures by around −2.0 kcal/mol. This value agrees with the estimated free energy of dimerization of the formic acid (around −3 kcal/mol).[63]

Table II also shows the free energy change arising upon proton transfer in the ionic complexes. The stability of both ionic and neutral complexes is similar for the symmetric (A, B) complexes. However, the neutral form is clearly more favored in the rest of structures. The neutral complex between methylamine and acetic acid is clearly more stable than the dimer formed by the corresponding ions. The calculated change in free energy for the proton transfer in the ionic complex is slightly more negative than the value reported for the complex between trimethylammonium and formate.[39]

TABLE II. Binding Free Energies[a] (kcal/mol) for Ionic and Neutral Complexes in the Gas Phase, and Free Energy Difference (ΔG_{PT}) Between Hydrogen-Bonded Ionic and Neutral Complexes

Structure[b]	Ionic	Neutral[c]	ΔG_{PT}[d]
	Methylguanidinium–acetate		
A	−105.6 (0.0)	−2.4 (0.0)	0.3
B	−104.3 (1.3)	−2.0 (0.4)	−0.5
C	−101.4 (4.2)	−1.8 (0.6)	−2.4
D	−99.4 (6.2)	−1.8 (0.6)	−6.3
E	−97.7 (7.9)	0.0 (2.4)	−4.8
F	−96.3 (9.3)	0.3 (2.7)	−5.3
	Methylammonium–acetate		
G	−112.9 (−7.3)	0.3 (2.7)	−7.8

[a]Zero-point, thermal and entropy (at 298 K) corrections were determined at the HF/6-31G(d) level. These corrections were added to the energy values in Table I in order to derive the free energy changes. Relative free energies are given in parentheses.
[b]See Figure 1.
[c]Binding free energies are computed relative to the most stable form of methylguanidine. Only the binding free energy of the most stable neutral complex arising upon transfer of hydrogen atoms 1 and 2 from methylguanidinium (methylammonium) to acetate is given.
[d]Free energy of proton transfer (PT) to form neutral complex from salt bridge.

TABLE III. Binding Free Energies[a] (kcal/mol) for Ionic and Neutral Complexes in Aqueous Solution, and Free Energy Difference (ΔG_{PT}) Between Hydrogen-Bonded Ionic and Neutral Complexes

Structure[b]	Ionic	Neutral[c]	ΔG_{PT}[d]
	Methylguanidinium–acetate		
A	12.2 (0.0)	3.3 (0.0)	18.7
B	12.5 (0.3)	3.5 (0.2)	18.7
C	13.4 (1.2)	4.8 (1.9)	18.1
D	13.9 (1.7)	3.8 (0.5)	16.5
E	14.5 (2.3)	2.0 (−1.3)	15.5
F	14.1 (1.9)	2.0 (−1.3)	16.5
	Methylammonium–acetate		
G	17.7 (5.5)	3.3 (0.0)	11.7

[a]Determined from addition of the change in the free energy of solvation upon dimerization to the free energy difference in the gas phase (see Table II). The changes in free energy for the dimerization in the gas phase were corrected to convert the values to a 1-M standard state. Relative values are given in parentheses.
[b]See Figure 1.
[c]See footnote c in Table II.
[d]Free energy of proton transfer (PT) to form neutral complex from salt bridge.

Calculations in Solution

The binding free energies, as well as the free energy difference between ionic and neutral complexes, in aqueous and chloroform solutions are reported in Tables III and IV. As expected, in all cases the complexes are less well solvated than their corresponding isolated monomers. For neutral complexes, this effect is mainly due to dipole annihilation of the monomers in the complex and to the loss

TABLE IV. Binding Free Energies[a] (kcal/mol) for Ionic and Neutral Complexes in Chloroform, and Free Energy Difference (ΔG_{PT}) Between Hydrogen-Bonded Ionic and Neutral Complexes

Structure[b]	Ionic	Neutral[c]	ΔG_{PT}[d]
	Methylguanidinium–acetate		
A	−27.9 (0.0)	−2.0 (0.0)	5.7
B	−27.1 (0.8)	−1.3 (0.7)	5.5
C	−24.1 (3.8)	−2.0 (0.0)	2.4
D	−23.3 (4.6)	−1.0 (0.8)	0.1
E	−21.3 (6.6)	−1.3 (0.7)	−0.2
F	−20.8 (7.1)	−1.2 (0.8)	−3.9
	Methylammonium–acetate		
G	−28.7 (0.8)	−0.3 (1.7)	1.6

[a]Determined from addition of the change in the free energy of solvation upon dimerization to the gas phase free energy difference (see Table II). The changes in free energy for the dimerization in the gas phase were corrected to convert the values to a 1M standard state. Relative values are given in parentheses.
[b]See Figure 1.
[c]Binding free energies are computed relative to the most stable form of methylguanidine. Only the binding energy of the most stable neutral complex arising upon transfer of hydrogen atoms 1 and 2 from methylguanidinium (methylammonium) to acetate is given.
[d]Free energy of proton transfer (PT) to form neutral complex from salt bridge.

of hydrogen-bonding capabilities, whereas for ionic species, it mainly stems from partial annihilation of the net charges upon complexation.

The solvent-induced destabilization of ionic complexes in water amounts to 112–120 and 132 kcal/mol for methylguanidininium and methylammonium, respectively (see Tables II and III). The difference in the free energy of solvation of the two cations can be realized from the larger charge concentration in the latter species. The formation of the ionic complex is strongly destabilized in all cases. In the case of neutral complexes, the change in free energy of solvation between the dimer and the monomers amounts to 4–8 kcal/mol, which makes the dimerization to be disfavored in water. The less destabilized structures are the twisted (E, F) complexes, which is due to the larger exposure of carboxyl and guanidine moieties to the solvent with regard to the symmetric and staggered structures. The destabilization of neutral complexes agrees with recent results for the dimerization of formic acid, which indicates that the hydrogen bond association is not stable in aqueous solution.[63] Similar findings have also been reported for the interaction between two molecules of N-methylacetamide.[65]

The destabilizing effect of chloroform, although less important than that of water, is significant even for neutral complexes (see Tables II and IV). Thus, the change in free energy of solvation between the dimer and the separated monomers amounts to around 1–2 and near 80 kcal/mol for neutral and ionic species, respectively. As a result, the binding of ionic species is still favored, which is in contrast with the results for aqueous solution (see Table III). Indeed, the formation of the doubly hydrogen-bonded neutral complex is favored by 1–2 kcal/mol for the interaction between acetic acid and methylguanidine; this process is slightly less favorable for the interaction with methylamine.

It is worth noting the effect of solvation on the proton transfer in ionic complexes. In water, even though the formation of both ionic and neutral complexes from the isolated monomers is not favored, the ionic complex is in all cases preferred over the neutral one, which reverts the trend found in the gas phase. In chloroform, the ionic interaction is still favored for the symmetric (A, B) structures. Such a preference is, nevertheless, greatly reduced for the less stable staggered (C, D) interactions or even reversed for the twisted (E, F) complexes.

In summary, our results suggest that the environment makes a decisive contribution to the binding free energy of ionic and neutral complexes, as well as to their relative stability. In the gas phase, the complexation process is greatly favored, and the neutral complex is generally more stable than the ion pair. High dielectric media do not stabilize either the hydrogen-bonded ionic or the neutral complexes owing to the large energetic penalty arising upon desolvation of the interacting monomers. The situation, nevertheless, is quite different in apolar or very low polar solvents, where not only the binding of ionic species, but also the dimerization of neutral monomers occurs. Indeed, the preference of the ion pair over the neutral complex is largely reduced or even reversed in very low dielectric media, where the relative stability between ionic and neutral complexes is expected to be sensibly affected by small changes in the permittivity of the medium. This finding, therefore, raises the question about the real nature of salt bridges in highly anisotropic media such as the interior of proteins.

Calculations in Proteins

Since a protein is not a homogeneous continuous dielectric system, the local environment surrounding each salt bridge can be greatly different, and the energetics of the interconversion between neutral and ionic forms of the complex can be strongly determined by such microenvironment. The direct extrapolation of the SCRF results to the study of salt bridges in proteins can, therefore, be criticized. Accordingly, we decided to examine this question by performing electrostatic calculations using X-ray crystal structures of proteins (see Methods). Several salt bridges between Arg and Asp or Glu residues were selected and classified according to their internal geometry in one of the structural types shown in Figure 1. The electrostatic contribution (ΔG_{tot}) to the

TABLE V. Electrostatic Contribution to the Binding Free Energy (kcal/mol) of Salt Bridges in Selected Proteins[a]

Protein	State	ΔG_{des}	ΔG_{dim}	ΔG_{prot}	ΔG_{tot}
		Structure A			
1cse	Ionic	18.4	−13.4	−1.7	3.2
	Neutral	11.3	−8.0	−1.0	2.2
2pck	Ionic	28.4	−17.9	0.9	11.4
	Neutral	16.5	−9.8	−0.3	6.4
2pck	Ionic	24.9	−16.0	1.9	10.8
	Neutral	12.9	−5.6	1.5	8.8
1hny	Ionic	13.7	−8.4	−2.8	2.5
	Neutral	8.6	−2.8	−1.5	4.2
2end	Ionic	12.1	−12.1	−0.8	−0.8
	Neutral	7.4	−5.7	−0.6	1.2
		Structure B			
1rro	Ionic	42.1	−26.5	−22.4	−6.8
	Neutral	22.3	−10.8	−14.6	−3.1
5p21	Ionic	2.4	−3.4	−5.3	−6.3
	Neutral	1.3	−1.3	−3.3	−3.3
1try	Ionic	13.0	−8.6	−7.0	−2.6
	Neutral	9.0	−5.5	−5.6	−2.1
1pda	Ionic	10.4	−9.2	−0.3	0.8
	Neutral	9.5	−5.9	−0.9	2.7
1pda	Ionic	14.8	−10.8	−4.0	0.0
	Neutral	10.8	−6.5	−3.1	1.2
		Structure F			
2pck	Ionic	18.8	−8.3	−8.8	1.7
	Neutral	9.5	−4.4	−4.6	0.5
1try	Ionic	14.8	−8.3	−7.2	−0.8
	Neutral	9.3	−1.0	−4.0	4.2
		Average[b]			
	Ionic	17.8 (10.2)	−11.9 (6.0)	−4.8 (6.5)	1.1 (5.6)
	Neutral	10.7 (5.1)	−5.6 (3.0)	−3.2 (4.1)	1.9 (3.7)

[a]Dielectric constants of 2 and 78.4 were defined for the interior of the protein and the solvent in these calculations.
[b]Standard deviation in parentheses.

binding of ionic and neutral monomers is given in Table V (see also Figure 2), which also shows the values computed for the desolvation of monomers (ΔG_{des}), the interaction between monomers in their position within the protein (ΔG_{dim}), and the interaction with the rest of residues in the protein (ΔG_{prot}). In these calculations, dielectric constants of 2 and 78.4 were defined for the interior of the protein and the solvent.

The results indicate that desolvation (ΔG_{des}) plays an important destabilizing effect, which ranges from 2 to 40 kcal/mol for the ionic species, whereas this energy penalty varies from 1 to 20 kcal/mol in the desolvation of the neutral monomers. Such a destabilizing effect is partially compensated by the electrostatic interaction between monomers (ΔG_{dim}), which leads to an attractive energy contribution ranging from −1 to −11 and from −3 to −26 kcal/mol for neutral and ionic complexes, respectively. The partial compensation between the energy cost due to desolvation of monomers and the energetic gain arising from their interaction in the folded protein is apparent in Figure 3. This can be understood from the fact that the desolvation energy will be larger as the burial of the interacting residues increases when the protein folds. Nevertheless, the coulombic interaction between the oppositely charged monomers in the complex will also increase owing to the reduced charge screening of the environment.

The interaction with the rest of residues (ΔG_{prot}) favors in general the formation of the complex, even though in a few cases its contribution is slightly destabilizing. The stabilization provided by the interaction with neighboring residues is, nevertheless, small in most cases. No relationship is observed between ΔG_{des} (or ΔG_{dim}) and ΔG_{prot} (see Fig. 4). This is not unexpected, since this latter term strongly depends on the local environment.[66,67] Thus, the Arg75–Glu81 complex in 1rro, which has the strongest value of ΔG_{prot} in Table V, establishes a network of hydrogen bonds with neighboring residues, particularly with the NH groups of Asp32 and Thr78, and

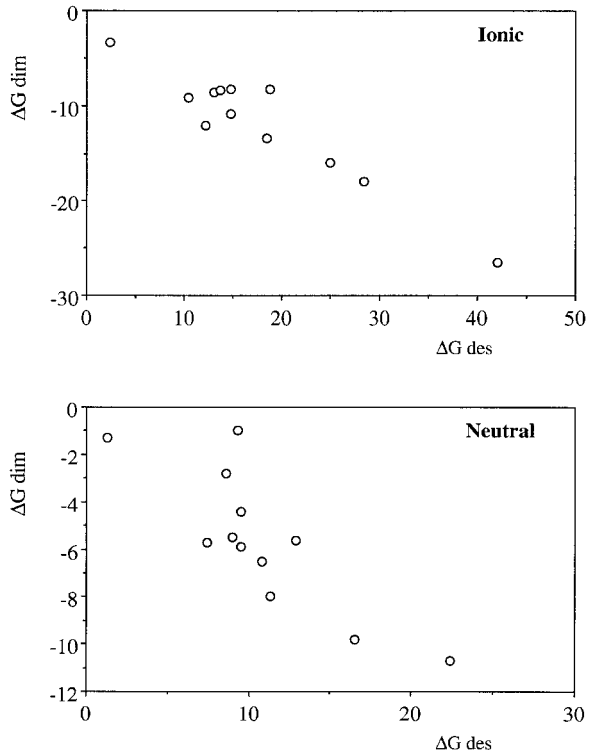


Fig. 3. Representation of the variation of ΔG_{dim} vs. ΔG_{des}. Values are in kcal/mol.

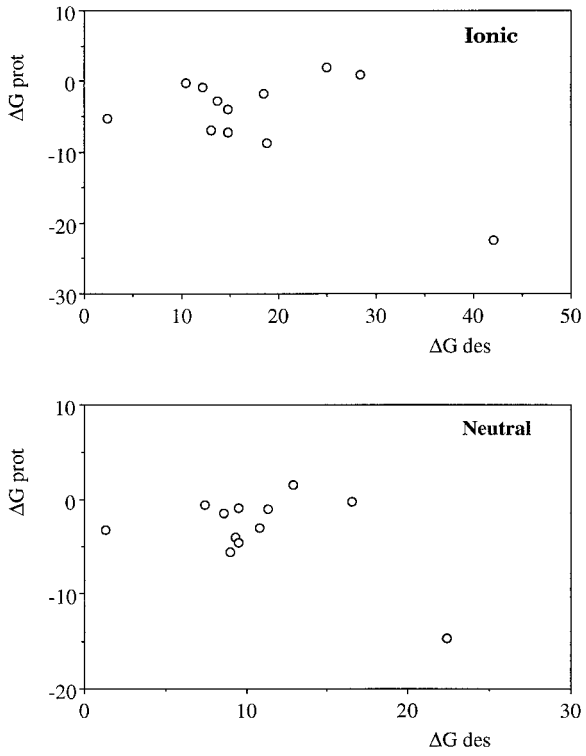


Fig. 4. Representation of the variation of ΔG_{prot} vs. ΔG_{des}. Values are in kcal/mol.

the carbonyl oxygen of Cys18. Likewise, all the type A complexes, for which ΔG_{prot} is very small or even destabilizing, form none or at most one hydrogen bond contact with neighboring residues. Obviously, in addition to the specific interactions with the closest residues, other factors such as the electrostatic field created by the atmosphere of charges and permanent dipoles surrounding the salt bridge complex and the solvent screening of electrostatic interactions also influence the magnitude of ΔG_{prot}.[68] This latter factor, for instance, explains the low value of ΔG_{prot} for the Arg123–Glu143 complex in 5p21, even though it establishes hydrogen bond interactions with residues Val125, Ser127, and Tyr141.

The formation of ionic and neutral complexes in the interior of proteins is determined from the balance between the preceding three contributions (ΔG_{tot} in Table V). In general, the formation of the complex is disfavored with regard to the state corresponding to the separated monomers in solution. Only in a few cases, such as the complexes formed between Arg75 and Glu81 in 1rro or between Arg123 and Glu143 in 5p21, the binding process is clearly favored. The results range between −7 and 11 kcal/mol for the interaction between ionic monomers, and between −3 and 9 kcal/mol for the neutral complexes. Moreover, there is no clear distinction in the electrostatic contribution between the different structural types. Comparison of the electrostatic free energies for the formation of neutral and ionic complexes (see Table V) reveals that, in general, the binding of neutral monomers is slightly less favored than the formation of the ion pair. On average, such a preference amounts to nearly 1 kcal/mol and stems mainly from the interactions with neighboring residues, which stabilize better the ion pair.

The preceding results were determined using a dielectric constant of 2 for the interior of proteins, which is the value of the high-frequency dielectric relaxation of most organic substances in the condensed phase. However, before discussing their biological implications, let us examine the dependence of ΔG_{tot} on the dielectric constant used for the interior of the protein. This is necessary since our model neglects some relevant microscopic effects, such as the reorganization energy of protein dipoles, which indeed is specific in each site within the protein, or the energy contribution from induced dipoles.[67,69] The results determined by using different permittivities are given in Table VI. The total electrostatic interaction exhibits a quite strong dependence on the interior dielectric constant. In general, increasing the permittivity reduces the stabilization for those favored contacts, but also makes less destabilizing those unfavored interactions. The influence of the dielectric constant is sensibly lower when the mean values are considered. Thus, the electrostatic interaction changes from 1.1 to −0.3 (ionic)

and from 1.9 to 0.2 (neutral) kcal/mol, when the permittivity varies from 2 to 8. Accordingly, the formation of the complex in the folded protein from the monomers in the unfolded state is not favored. Nevertheless, these findings must be taken in an "average" sense, since both the standard deviation of the mean values and the results in Table V emphasize that the binding is greatly influenced by the local microenvironment.

From the preceding results, we can now try to answer two relevant questions about the nature and the role of salt bridges in proteins. First, what is the stability between the ionic and neutral forms of salt bridges and how does it compare with the results determined in solution? Second, what is the contribution of salt bridges to the stability of proteins?

The relative stability of the ionic and neutral hydrogen-bonded forms of the Arg–Asp(Glu) salt bridges in the interior of proteins can be determined from the thermodynamic cycle shown in Figure 5. According to this scheme, the free energy change for the proton transfer in the ion pair can be estimated by adding the difference between the free energy of formation of neutral and ionic complexes in the folded protein ($\Delta G_{tot,neutral} - \Delta G_{tot,ionic}$) to the free energy difference between the neutral and ionic states of the separated monomers in the unfolded state (ΔG_1). The term $\Delta G_{tot,neutral} - \Delta G_{tot,ionic}$ can be reasonably approximated by the electrostatic free energy difference, which is the most important contribution, since other components are expected to cancel roughly between the ionic and neutral complexes. Indeed, assuming that the ionizable groups of the residues in the unfolded state are well exposed to the solvent and that their intrinsic acid/base properties are not substantially affected by the polypeptide chain, this term ΔG_1 can be estimated from the pK_a values of the guanidinium and carboxyl moieties in Arg and Asp(Glu).

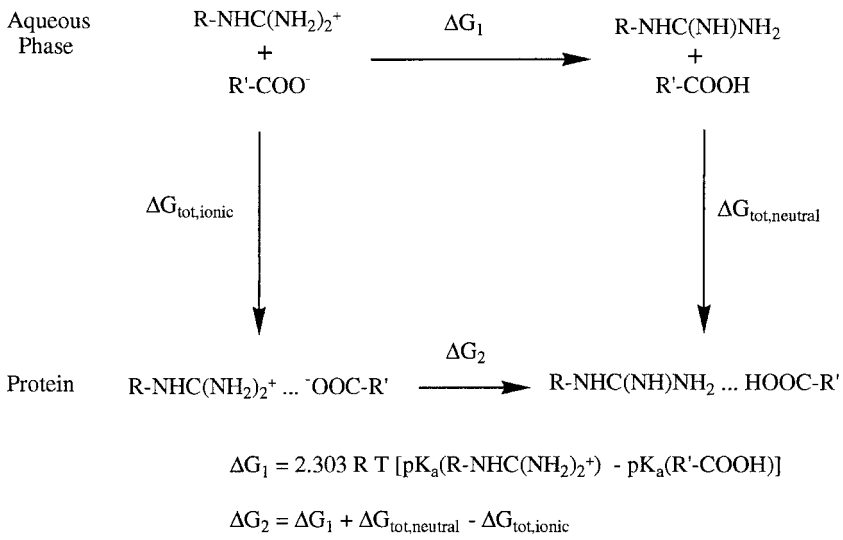
By using the values of $\Delta G_{tot,neutral}$ and $\Delta G_{tot,ionic}$ given in Table V, the ion pair formed between the guanidinium and carboxylate groups is found to be, on average, more stable than the neutral complex by 12.4 ± 2.8 kcal/mol (similar estimates are obtained when the results determined for $\epsilon = 4$ and $\epsilon = 8$ are used). This value lies between the free energy differences of ionic and neutral complexes in water and in chloroform (see Tables III and IV), which a priori suggests the definition of an effective permittivity to simulate the dielectric relaxation response of the proteins in SCRF continuum calculations. However, it must be stressed that the free energy differences between the ionic and neutral complexes for the individual methylguanidium–acetate structures in the proteins range from 6.6 to 16.6 kcal/mol. The high diversity in the values both emphasizes the difficulty to assign a unique value of dielectric constant for the local environment in proteins, whose anisotropic nature has to be explicitly considered, and argues against the direct extrapolation of SCRF results to the study of these interactions in proteins.

With regard to the contribution of salt bridges to the stability of proteins, our results indicate that the formation of an ion pair contributes to the protein stability less than 1 kcal/mol with regard to the interaction between neutral groups, even though the formation of both ionic and neutral complexes from

TABLE VI. Dependence of the Electrostatic Free Energies (kcal/mol) for the Formation of Ionic and Neutral Complexes in the Protein on the Dielectric Constant for the Interior of the Protein

Protein	State	$\epsilon = 2$	$\epsilon = 4$	$\epsilon = 8$
		Structure A		
1cse	Ionic	3.2	1.2	−0.1
	Neutral	2.2	0.9	0.1
	$\Delta\Delta G_{tot}$[a]	−1.0	−0.3	−0.2
2pck	Ionic	11.4	5.1	1.9
	Neutral	6.4	2.7	1.0
	$\Delta\Delta G_{tot}$	−5.0	−2.4	−0.9
2pck	Ionic	10.8	4.8	4.2
	Neutral	8.8	4.1	3.7
	$\Delta\Delta G_{tot}$	−2.0	−0.7	−0.5
1hny	Ionic	2.5	0.5	−0.4
	Neutral	4.2	1.8	0.6
	$\Delta\Delta G_{tot}$	1.7	1.3	1.0
2end	Ionic	−0.8	−1.1	−1.2
	Neutral	1.2	0.3	−0.1
	$\Delta\Delta G_{tot}$	2.0	1.4	1.1
		Structure B		
1rro	Ionic	−6.8	−4.4	−3.2
	Neutral	−3.1	−2.2	−1.7
	$\Delta\Delta G_{tot}$	3.7	2.2	1.5
5p21	Ionic	−6.3	−2.3	−1.2
	Neutral	−3.2	−0.8	−0.5
	$\Delta\Delta G_{tot}$	3.1	1.5	0.7
1try	Ionic	−2.6	−1.7	−1.3
	Neutral	−2.1	−1.2	−0.8
	$\Delta\Delta G_{tot}$	0.5	0.5	0.5
1pda	Ionic	0.8	−0.1	−0.6
	Neutral	2.7	1.1	0.4
	$\Delta\Delta G_{tot}$	1.9	1.2	1.0
1pda	Ionic	0.0	−0.4	−0.6
	Neutral	1.2	0.4	−0.1
	$\Delta\Delta G_{tot}$	1.4	0.8	0.7
		Structure F		
2pck	Ionic	1.7	0.0	−0.7
	Neutral	0.5	−0.4	−0.3
	$\Delta\Delta G_{tot}$	−1.2	−0.4	0.4
1try	Ionic	−0.8	−0.9	−1.0
	Neutral	4.2	1.9	0.7
	$\Delta\Delta G_{tot}$	5.0	2.8	1.7
		Average[b]		
	Ionic	1.1 (5.6)	0.1 (2.7)	−0.3 (0.8)
	Neutral	1.9 (3.7)	0.7 (1.8)	0.2 (1.4)
	$\Delta\Delta G_{tot}$	0.8 (2.6)	0.6 (1.4)	0.5 (0.8)

[a] $\Delta G_{tot,neutral} - \Delta G_{tot,ionic}$.
[b] Standard deviation in parentheses.



$$\Delta G_1 = 2.303\ R\ T\ [pK_a(R\text{-}NHC(NH_2)_2^+) - pK_a(R'\text{-}COOH)]$$

$$\Delta G_2 = \Delta G_1 + \Delta G_{tot,neutral} - \Delta G_{tot,ionic}$$

Fig. 5. Thermodynamic cycle for the formation of ionic and neutral complexes in the interior of the protein from the infinitely separated monomers in the unfolded state.

the separated monomers in the unfolded state is, on average, not particularly favored. The contribution of salt bridges to the protein stability has been estimated experimentally to be in the range of 1–5 kcal/mol.[70–72] However, the direct comparison with our results is not pertinent, since most of the available data were determined by measuring pK_a shifts or from mutation to polar or apolar residues. Therefore, the stabilization due to the formation of the salt bridge in the folded protein is measured relative to one or more charged hydrogen bonds,[31] whereas our estimates of $\Delta\Delta G_{tot}$ (see Table VI) correspond to the stability arising upon formation of the ion pair relative to the neutral hydrogen-bonded complex. A more reliable comparison is afforded from double mutant experiments, where the salt bridge residues are mutated to neutral polar species. For instance, the coupling energy for the bridges Arg69–Asp93 and Arg83–Asp75 in barnase has recently been estimated to be around 3 kcal/mol.[70] Even in these cases caution is required, since the mutation can affect packing interactions or induce conformational changes, which are not treated in an explicit way in our study.

The finding that the formation of an ion pair is slightly more favored than the formation of the neutral complex in proteins is not in contradiction with recent studies that have questioned the contribution of salt bridges to the stability of proteins.[31,34,71] In these studies, the stabilizing contribution of the salt bridge interaction was measured relative to variants of the protein containing hydrophobic isosteres. Both theoretical and experimental data indicate that purely hydrophobic interactions contribute more to the protein stability than the salt bridge interaction. This difference was estimated from theoretical calculations to be, on average, around 3.5 kcal/mol.[34] According to our calculations, the substitution of hydrogen bond complexes by residues forming hydrophobic contacts is expected to be, on average, even more stabilizing.

The favorable interaction between the monomers for some particular salt bridges (see Table V) suggests that in some cases the ion pair can contribute significantly to the stability of the folded protein. However, the formation of the complex does not lead in general to a net stabilizing effect, and mutation by hydrophobic isosteres can lead to stabilization of the protein.[31,34] Therefore, it can be speculated that biological relevance of salt bridges might be related to roles other than the thermodynamic stabilization of the folded state. Present results give support to the idea that ion pairs, as well as neutral hydrogen-bonded complexes, contribute to the protein structure by providing "specificity" rather than a significant energetic stabilization. The existence of these interactions in the protein might stem from a subtle compromise between the loss in stabilization energy, which would be more favorable upon replacement by hydrophobic residues, and the gain in specificity inherent to electrostatics and hydrogen bond interactions, which do not exist in case of interactions between hydrophobic residues.

According to the principle of compensation between energy and specificity, this kind of interactions might play a decisive role in guiding the folding pathway, so that a buried salt link could kinetically direct the polymer folding by limiting substantially the exploration of all the allowable foldings. The role of salt bridges would, then, be not to stabilize the protein thermodynamically but to guide the first early steps in the folding process or to play a kinetic

control in the folding pathway.[71] This idea can also be extended to the role played by networks of hydrogen bonds. Even though their contribution to the stabilization is expected to be lower than for hydrophobic interactions, the directionality of the hydrogen bond would reduce strongly the number of potential arrangements in joining different structural domains or by limiting the conformational degree of freedom. Similar considerations could also be made to the recognition and binding of substrates mediated by salt bridges.

In addition to the preceding discussion about the contribution to stability, another interesting aspect concerns the formation of ion pairs in the reaction pathway catalyzed by enzymes, which has been examined in detail by Warshel.[30,73,74] In this case, the enzyme has been found to provide substantial stabilization of the ionic transition state. It was concluded that enzymes are designed to accelerate reactions owing to the fact that the polar active site stabilizes an ionic transition state better than water can. The polar environment at the active site can then be viewed as a supersolvent by providing optimal solvation to the ionic forms at the transition state. To achieve this, the protein pays folding energy to arrange the polar groups in a relatively high-energy configuration that gives such an optimal stabilization.

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REFERENCES

1. Barlow, D.J., Thornton, J.M. Ion pairs in proteins. J. Mol. Biol. 168:867–885, 1983.
2. Riordan, J.F., McElvany, R.D., Borders, C.L. Arginyl residues: Anion recognition sites in enzymes. Science 195:884–886, 1977.
3. Kirsch, J.F., Eichele, G., Ford, G.C., et al. Mechanism of action of aspartate aminotransferase proposed on the basis of its spatial structure. J. Mol. Biol. 174:497–525, 1984.
4. Singh, J., Thornton, J.M., Snarey, M., Campbell, S.F. The geometry of interacting arginine–carboxyl in proteins. FEBS Lett. 224:161–171, 1987. See erratum in FEBS Lett. 228:357, 1988.
5. Tormo, J., Blaas, D., Parry, N.R., Rowlands, D., Stuart, D., Fita, I. Crystal structure of a human rhinovirus neutralizing antibody complexed with a peptide derived from viral capsid protein VP2. EMBO J. 13:2247–2256, 1994.
6. Raumann, B.E., Rould, M.A., Pabo, C.O., Sauer, R.T. DNA recognition by β-sheets in the Arc repressor–operator crystal structure. Nature 367:754–757, 1994.
7. Brown, B.M., Milla, M.E., Smith, T.L., Sauer, R.T. Scanning mutagenesis of the Arc repressor as a functional probe of operator recognition. Nature Struct. Biol. 1:164–168, 1994.
8. Perutz, M.F. Mechanisms of cooperativity and allosteric regulation in proteins. Q. Rev. Biophys. 22:139–236, 1989.
9. Schirmer, T.E., Evans, P.R. Structural basis of the allosteric behavior of phosphofructokinase. Nature 343:140–145, 1990.
10. Gandini, D., Gogioso, L., Bolognesi, M., Bordo, D. Patterns of ionizable sidechains interactions in protein structures. Proteins 24:439–449, 1996.
11. Marqusee, S., Baldwin, R.L. Helix stabilization by Glu^{-} . . . Lys^{+} salt bridges in short peptides of de novo design. Proc. Natl. Acad. Sci. U.S.A. 84:8898–8902, 1987.
12. Huyghues-Despointes, B.M.P., Baldwin, R.L. Ion pair and charged hydrogen bond interactions between histidine and aspartate in a peptide helix. Biochemistry 36:1965–1970, 1997.
13. Lyu, P.C., Gans, P.J., Kallenbach, N.R. Energetic contribution of solvent-exposed ion pairs to alpha-helix structure. J. Mol. Biol. 223:343–350, 1992.
14. Sundaralingam, S., Sekhadrudu, Y.C., Yathindra, N., Ravichadran, V. Ion pairs in alpha helices. Proteins 2:64–71, 1987.
15. Dym, O., Mevarech, M., Sussman, J.L. Structural features that stabilize halophilic malate–dehydrogensase from an archaebacterium. Science 267:1344–1346, 1995.
16. Argos, P. An investigation of protein subunit and domain interfaces. Protein Eng. 2:101–113, 1988.
17. Janin, J., Miller, S., Clothia, C. Surface subunit interfaces and interior of oligomeric proteins. J. Mol. Biol. 204:1551–1564, 1988.
18. Musafia, B., Buchner, V., Arad, D. Complex salt bridges in proteins: Statistical analysis of structure and function. J. Mol. Biol. 254:761–770, 1995.
19. Ward, W.H.J., Hugh Jones, D., Fersht, A.R. Effects of engineering complementary charged residues into the hydrophobic subunit interface of tyrosyl-tRNA synthetase. Biochemistry 26:4131–4138, 1987.
20. Fersht, A.R. Conformational equilibria in α- and δ-chymotrypsin. The energetics and importance of the salt bridge. J. Mol. Biol. 64:497–509, 1972.
21. Horovitz, A. The relation between cooperativity in ligand binding and intramolecular cooperativity in allosteric proteins. Proc. Roy. Soc. Ser. B259:85–87, 1995.
22. Horovitz, A., Serrano, L., Avron, B., Bycroft, M., Fersht, A.R. Strength and cooperativity of contributions of surface salt bridges to protein stability. J. Mol. Biol. 216:1031–1044, 1990.
23. Oliveberg, M., Fersht, A.R. A new approach to the study of transient protein conformations: The formation of a semi-buried salt link in the folding pathway of barnase. Biochemistry 35:6795–6805, 1996.
24. Ruegg, C., Ammer, D., Lerch, K. Comparison of amino acid sequence and thermostability of tyrosinase from three wild-type strains of *Neurospora crassa*. J. Biol. Chem. 257:6420–6426, 1982.
25. Kelly, C.A., Nishiyama, M., Shanishi, Y., Beppu, T., Birktoft, J.J. Determinants of protein stability observed in the 1.9-Å crystal structure of malate dehydrogensase from the thermophilic bacterium *Thermus falavus*. Biochemistry 32:3913–3922, 1993.
26. Dao-Pin, S., Sauer, U., Nicholson, H., Matthews, B.W. Contributions of engineered surface salt bridges to the stability of T4 lysozyme determined by directed mutagenesis. Biochemistry 30:7142–7153, 1991.
27. Serrano, L., Horovitz, A., Avron, B., Bycroft, M., Fersht, A.R. Estimating the contribution of engineered surface electrostatic interactions to protein stability by using double-mutant cycles. Biochemistry 29:9343–9352, 1990.
28. De Prat Gay, G., Johnson, C.M., Fersht, A.R. Contribution of a proline residue and a salt bridge to the stability of a type I reverse turn in chymotripsin inhibitor-2. Protein Eng. 7:103–108, 1994.
29. Sali, D., Bycroft, M., Fersht, A.R. Surface electrostatic interactions contribute little to stability of barnase. J. Mol. Biol. 220:779–788, 1991.
30. Warshel, A., Russell, S.T., Churg, A.K. Macroscopic models for studies of electrostatic interactions in proteins: Limitations and applicability. Proc. Natl. Acad. Sci. U.S.A. 81: 4785–4789, 1984.
31. Waldburger, C.D., Schildbach, J.F., Sauer, R.T. Are buried salt bridges important for protein stability and conformational specificity? Nature Struct. Biol. 2:122–128, 1995.
32. Dao-Pin, S., Anderson, D.E., Baase, W.A., Dahlquist, F.W.,

Matthews, B.W. Structural and thermodynamic consequences of burying a charged residue within the hydrophobic core of T4 lysozime. Biochemistry 30:11521–11529, 1991.
33. Wimley, W.C., Gawrisch, K., Creamer, T.P., White, S.H. Direct measurement of salt bridge solvation energies using a peptide model system: Implications for protein stability. Proc. Natl. Acad. Sci. U.S.A. 93:2985–2990, 1996.
34. Hendsh, Z.S., Tidor, B. Do salt bridges stabilize proteins? A continuum electrostatic analysis. Prot. Sci. 3:211–226, 1994.
35. Honig, B.H., Hubbell, W.L. Stability of "salt bridges" in membrane proteins. Proc. Natl. Acad. Sci. U.S.A. 81:5412–5416, 1984.
36. Warshel, A. Electrostatic basis of structure–function correlation in proteins. Acc. Chem. Res. 14:284–290, 1981.
37. Warshel, A. Calculations of enzymatic reactions: Calculations of pK_a, proton transfer reactions, and general acid catalysis reactions in enzymes. Biochemistry 20:3167–3177, 1981.
38. Zheng, Y.-J., Ornstein, R.L. What happens to salt bridges in nonaqueous environments: Insights from quantum mechanics calculation. J. Am. Chem. Soc. 118:11237–11243, 1996.
39. Liljefors, T., Norrby, P.O. An ab initio study of the trimethylamine–formic acid and the trimethylammonium ion–formate anion complexes, their monohydrates, and continuum solvation. J. Am. Chem. Soc. 119:1052–1058, 1997.
40. Mitchell, J.B.O., Thornton, J.M., Singh, J., Price, S.L. Towards an understanding of the arginine–aspartate interaction. J. Mol. Biol. 226:251–262, 1992.
41. Hariharan, P.C., Pople, J.A. The influence of polarization functions on molecular orbital hydrogenation energies. Theor. Chim. Acta. 28:213–222, 1973.
42. Frisch, M.J., Pople, J.A., Binkley, J.S. Self-consistent molecular orbital methods. 25. Supplementary functions for Gaussian basis sets. J. Chem. Phys. 80:3265–3269, 1984.
43. Boys, S.F., Bernardi, F. The calculation of small molecular interactions by the difference of separate total energies: Some procedures with reduced errors. Mol. Phys. 19:553–566, 1970.
44. Frisch, M.J., Trucks, G.W., Schelgel, H.B., et al. Gaussian-94 (Rev. A.1). Pittsburg, PA: Gaussian Inc., 1995.
45. Bachs, M., Luque, F.J., Orozco, M. Optimization of solute cavities and van der Waals parameters in ab initio MST-SCRF calculations of neutral molecules. J. Comput. Chem. 15:446–454, 1994.
46. Orozco, M., Bachs, M., Luque, F.J. Development of optimized MST/SCRF methods for semiempirical calculations: The MNDO and PM3 hamiltonians. J. Comput. Chem. 16:563–575, 1995.
47. Luque, F.J., Zhang, Y., Aleman, C., Bachs, J., Gao, J., Orozco, M. Solvent effects in chloroform solution: Parametrization of the MST/SCRF continuum model. Phys. Chem. 100:4269–4276, 1996.
48. Miertus, S., Scrocco, E., Tomasi, J. Electrostatic interaction of a solute with a continuum: A direct utilization of ab initio molecular potentials for the prevision of solvent effects. Chem. Phys. 55:117–129, 1981.
49. Tomasi, J., Persico, M. Molecular interactions in solution: An overview of methods based on continuous distributions of the solvent. Chem. Rev. 94:2027–2094, 1994.
50. Pierotti, R. A scaled particle theory of aqueous and nonaqueous solutions. Chem. Rev. 76:717–726, 1976.
51. Orozco, M., Luque, F.J. Optimization of the cavity size for ab initio MST/SCRF calculations of monovalent ions. Chem. Phys. 182:237–248, 1994.
52. Bernstein, F.C., Koetzle, T.F., Williams, G.J.B., et al. The Protein Data Bank: A computer-based archival file for macromolecular structures. J. Mol. Biol. 112:535–542, 1977.
53. Gilson, M.K., Honig, B.H. Calculation of the total electrostatic energy of a macromolecular system: Solvation energies, binding energies, and conformational analysis. Proteins 4:7–18, 1988.
54. Gilson, M.K., Sharp, K.A., Honig, B.H. Calculating the electrostatic potential of molecules in solution: Method and error assessment. J. Comput. Chem. 9:327–335, 1988.
55. Pearlman, D.A., Case, D.A., Caldwell, J.C., et al. AMBER 4.0. San Francisco: University of California, 1991.
56. Peterson, M., Poirier, R. MonsterGauss. Toronto, Canada: University of Toronto. Modified by Cammi, R., Bonaccorsi, R., Tomasi, J., Pisa, Italy: University of Pisa, 1987. Modified by Luque, F.J., Orozco, M. Barcelona, Spain: University of Barcelona, 1995.
57. Insight-II. San Diego, CA: Biosym Technologies, 92121–2777.
58. Warshel, A. Calculations of chemical processes in solutions. J. Phys. Chem. 83:1640–1652, 1979.
59. Sapse, A.M., Russell, C.S. Theoretical studies of the binding of methylamine and guanidine to carboxylate. J. Mol. Struct. (Theochem) 137:43–53, 1986.
60. Deerfield, D.W., Nicholas, H.B., Jr., Hiskey, R.G., Pedersen, L.G. Salt or ion bridges in biological systems: A study employing quantum and molecular mechanics. Proteins 6:168–192, 1989.
61. Mavri, J., Vogel, H.J. Ion pair formation involving methylated lysine side chains: A theoretical study. Proteins 18:381–389, 1994.
62. Melo, A., Ramos, M.J. Proton transfer in arginine–carboxylate interactions. Chem. Phys. Letters 245:498–502, 1995.
63. Colominas, C., Teixidó, J., Cemelí, J., Luque, F.J., Orozco, M. Dimerization of carboxylic acids: Reliability of theoretical calculations and the effect of solvent. J. Phys. Chem. In press.
64. Heidrich, D., van Eikema Hommes, N.J.R., von Ragué Schleyer, P. Ab initio models for multiple-hydrogen exchange: Comparison of cyclic four- and six-center systems. J. Comput. Chem. 14:1149–1163, 1993.
65. Jorgensen, W.L. Interactions between amides in solution and the thermodynamics of weak binding. J. Am. Chem. Soc. 111:3770–3771, 1989.
66. Hwang, J.-K., Warshel, A. Why ion pair reversal by protein engineering is unlikely to succeed? Nature 334:270–273, 1988.
67. Sham, Y.K., Chu, Z.T., Warshel, A. Consistent calculations of pKas of ionizable residues in proteins: Semimicroscopic and microscopic approaches. J. Phys. Chem. B. 107:4458–4472, 1997.
68. Warshel, A., Russell, S.T. Calculations of electrostatic interactions in biological systems and in solution. Q. Rev. Biophys. 17:283–422, 1984.
69. Warshel, A., Åqvist, J. Electrostatic energy and macromolecular function. Ann. Rev. Biophys. Biophys. Chem. 20:267–298, 1991.
70. Tissot, A.C., Vuilleumeier, S., Fersht, A.R. Importance of two buried salt bridges in the stability and folding pathway of barnase. Biochemistry 35:6786–6794, 1996.
71. Marqusee, S., Sauer, R.T. Contributions of a hydrogen bond/salt bridge network to the stability of secondary and tertiary structure in λ repressor. Prot. Sci. 3:2217–2225, 1994.
72. Anderson, D.E., Becktel, W.J., Dahlquist, F.W. pH-induced denaturation of proteins: A single salt bridge contributes 3–5 kcal/mol to the free energy of folding of T4 lysozyme. Biochemistry 29:2403–2408, 1990.
73. Åqvist, J., Warshel, A. Simulation of enzyme reactions using valence bond force fields and other hybrid quantum/classical approaches. Chem. Rev. 93:2523–2544, 1993.
74. Warshel, A. "Computer Simulation of Chemical Reactions in Enzymes and Solution." New York: John Wiley & Sons, Inc., 1991.