Protein Science (1997), 6:2477–2482. Cambridge University Press. Printed in the USA.

FOR THE RECORD

The partial charge of the nitrogen atom in peptide bonds

E. JAMES MILNER-WHITE

Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, Glasgow University, Glasgow G12 8QQ, United Kingdom

(RECEIVED June 12, 1997; ACCEPTED August 13, 1997)

Abstract: A majority of the standard texts dealing with proteins portray the peptide link as a mixture of two resonance forms, in one of which the nitrogen atom has a positive charge. As a consequence, it is often believed that the nitrogen atom has a net positive charge. This is in apparent contradiction with the partial negative charge on the nitrogen that is used in force fields for molecular modeling. However, charges on resonance forms are best regarded as formal rather than actual charges and current evidence clearly favors a net negative charge for the nitrogen atom. In the course of the discussion, new ideas about the electronic structure of amides and the peptide bond are presented.

Keywords: α-helix; amides; electrostatics; hydrogen bonds; proteins; quantum mechanics

This article begins with a listing of some estimates of the partial charges of the atoms of amides. The planarity of amides is typically explained by portraying them as two resonance forms, one of which has a nitrogen atom with a formal positive charge. However, it is chemically unsatisfactory for the nitrogen atom to have a net partial positive charge, and the reasons for this are given. The strongest evidence that the nitrogen is negative rather than positive comes from quantum mechanics. Some recent work on amides and its implications for the electronic structure of the σ and π orbitals of the CONH group are discussed in some detail. The effect of hydrogen bonding is then considered and, finally, the relevance of these insights for α-helices is addressed.

Point charges on individual atoms: The resonance method used commonly to represent the peptide bond as in Figure 1 is often taken to imply that the nitrogen has a partial positive charge. This conflicts with the partial charges used widely in molecular mechanics force fields for modeling biological molecules. A set of calculated partial charges has been collected (Rogers, 1986), and a more up-to-date set is given in Table 1. In all cases, the nitrogen atom has a partial negative charge. The precise value of this partial charge has been debated extensively (Weiner et al., 1984; Cornell et al., 1995). Different authors use different methods, some based on quantum mechanics and some on semi-empirical calculations; they can give rise to substantially different sets of figures, as is evident in Table 1. Another source of variability is that the charges are likely to vary with conformation and environment (Reynolds et al., 1992). However, the various calculations do all give rise to negatively charged nitrogens and we shall be concerned with their signs more than the absolute values.

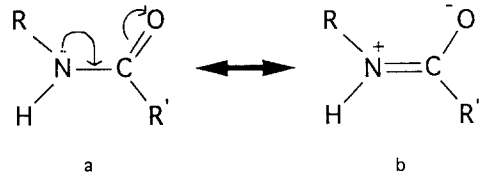

Fig. 1. Major resonance forms of the peptide bond as presented in many standard texts.

Explaining amide planarity: The high stability of the peptide bond derives from its being an amide. Amides are mostly found to be planar (in the sense that the two atoms of the C-N bond plus the four atoms covalently bonded to them are coplanar), although the barrier to rotation is not high (Dunitz & Winkler, 1983; Gilli et al., 1986; Wiberg & Laidig, 1987) and *cis* to *trans* isomerization occurs fairly rapidly. Almost all peptide bonds between amino acids in proteins in the Brookhaven Protein Data Bank are planar, with an average value for the dihedral angle ω of 179.5 ± 3.8° (Karplus, 1996). This could be due to a lack of resolution combined with the restraint toward planarity in the refinement programs used by protein crystallographers. The restraint does not enforce total planarity, but it does impose a bias favoring it. A higher proportion of the small polypeptides in the Cambridge Crystallographic Database, which are at higher resolution and not subject to the same restraints in the refinement process as the protein structures, exhibit significantly nonplanar peptide bonds. The crystal structure of acetamide

Reprint request to: E. James Milner-White, Division of Biochemistry and Molecular Biology, Institute of Biomedical and Life Sciences, Glasgow University, Glasgow G12 8QQ, United Kingdom; e-mail: j.milner-white@bio.gla.ac.uk.

Table 1. *Partial charges on atoms in the peptide link*[a]

		DSSP	AMBER	2ndGEN	6-311++G**
C	Carbonyl carbon	+0.42	+0.50	+0.60	+1.48
O	Carbonyl oxygen	−0.42	−0.53	−0.57	−1.11
N	NH nitrogen	−0.20	−0.52	−0.42	−1.23
H	NH hydrogen	+0.20	+0.25	+0.27	+0.42

[a]The DSSP column shows the charges used in the DSSP (Dictionary of Secondary Structure in Proteins) program (Kabsch & Sander, 1983) for estimating hydrogen bond energies; the charges are derived from semi-empirical calculations (Poland & Scheraga, 1967). The middle columns give the charges used in the force fields AMBER (Weiner et al., 1984) and 2ndGEN (Cornell et al., 1995), a second generation force field designed as successor to AMBER. AMBER uses the STO-3G basis set for electrostatic potential (ESP) fit charge derivation; 2ndGEN uses the 6-31G* basis set for derivation of restrained ESP-fit partial charges. The column at the right gives partial charges from ab initio calculations (Wiberg et al., 1992) at the MP2/6-311++G** level for formamide.

has been studied by neutron diffraction (Jeffrey et al., 1980). It is fairly planar, but not totally so, the two O-C-N-H dihedral angles being 179.5° and −8°.

To explain this planarity, the majority of books, both chemical and biochemical, dealing with proteins (Fersht, 1985; Darnell et al., 1986; Fessenden & Fessenden, 1986; Bailey, 1990; Ege, 1990; Voet & Voet, 1990; Glusker et al., 1991; McMurray, 1991; Abeles et al., 1992; Lehninger et al., 1992; Zubay, 1993; Hamaguchi, 1994; Solomons, 1994; Palmer, 1995; Stryer, 1995; Zubay et al., 1995; Chang, 1996; Mathews & van Holde, 1996; Murray et al., 1996; Elliott & Elliott, 1997; Fox & Whitesell, 1997) present amides as a mixture of the two resonance forms in Figure 1 leaving the impression for many that the nitrogen atom has a positive charge. Three worthy exceptions are those by Creighton (1993) and Branden and Tooze (1991), which give the "DSSP" partial charges in Table 1, and Jeffrey and Saenger's book in 1991, which tabulates sets of ab initio-derived charges for amides (with negative nitrogen atoms), although elsewhere has illustrations of peptides with positively charged nitrogens. According to the standard resonance representation, there is a transfer of electrons from the nitrogen to the carbonyl oxygen atom. A π-orbital forms, as in species 1b, between the carbon and nitrogen atoms, resulting in shortening of the C-N bond and coplanarity of the six atoms involved. The nitrogen of species 1b develops a formal positive charge, and readers are liable to gather that the reason for amide planarity is that negative charge is transferred from N to C to O.

Although the valency bond/resonance method is useful for giving some idea what sort of bonds may exist, it is not meant (Webster, 1990) to be effective at predicting partial atomic charges. This is why the charges so designated are often called formal charges. The positive nitrogen atom of species 1b might have been expected to make this atom partially positive in amides as a whole. Presumably, however, the NH hydrogen atom is also positive, because hydrogen is more electropositive than nitrogen, yet the evidence is that a proton is not lost easily (the pK_a of formamide is 17.2) (Homer & Johnson, 1970; Perrin, 1994). Nor is there significant transfer of the NH proton by tautomerization, the equilibrium constant for interconversion (Fersht, 1971) of an amide, C(=O)-NH, and its tautomer imidic acid, C(OH)=N, being 10^{-8}.

An observation in accord with this comes from the planarity of X-Pro peptide bonds. They have no hydrogen atom that might be lost, yet are no less planar (the average value for ω is 179.6 Å; Karplus, 1996) than the other peptide bonds. Another point is that the average value of the N-C-O bond angle in proteins is 123° (Karplus, 1996); this is consistent with repulsion between partly negative nitrogen and oxygen atoms, causing the angle to increase above the expected value of 120°. Lastly, it seems intuitively unlikely that three atoms (C, N, and H) covalently bonded to each other in a particularly stable atomic grouping would all have partial positive charges.

What quantum mechanics reveals: Quantum mechanical calculations on amides, showing the nitrogen atom to be negative rather than positive, were performed more than 30 years ago (Poland & Scheraga, 1967). In general, quantum mechanical estimates of partial charges are thought to be correct because they agree well (Gatti et al., 1992) with those derived from high-resolution charge density crystallographic studies performed at low temperatures on small molecules, at least for nontransition metal-containing molecules.

Ab initio calculations on amides (Wiberg & Laidig, 1987; Laidig & Bader, 1991; Wiberg & Breneman, 1992; Wiberg & Glaser, 1992; Wiberg et al., 1992; Wiberg & Rablen, 1993, 1995; Laidig & Cameron, 1996) have been performed, starting with the planar forms (with nitrogens sp^2-hybridized), and allowing them to rotate about the C-N bond such that they change to forms with sp^3-hybridized pyramidal nitrogen atoms; these studies show that the degree of negative charge on the carbonyl oxygen is little affected by the rotation process; furthermore, there is only a small change in the distance between the carbon and oxygen of the carbonyl group. This appears inconsistent with the scheme in Figure 1, where both the negative charge on the oxygen and the C-O distance would be expected to be relatively high in the planar form and should decrease substantially going to a pyramidalized form.

The same set of calculations do, however, reveal substantial changes at the C-N bond during this change in conformation. On rotating away from planarity toward the tetrahedral species, the C-N distance increases, and, strikingly, there there is a net electron transfer from the nitrogen to the carbonyl carbon atom. When the molecule becomes planar again, the transfer of electrons is to the nitrogen from the carbonyl carbon atom. These *net* electron transfers are in the opposite directions from those implied by the arrows in Figure 1A, and are consistent with the nitrogen atom becoming more, rather than less, negative in the planar form and certainly not with it becoming at all positive.

A nitrogen atom with a partial negative charge is to be expected with the relatively electronegative nitrogen atom attracting electrons toward itself from adjacent atoms. In spite of the p-orbital electrons of the nitrogen being shared with the carbonyl carbon atom, the electronegativity of the nitrogen is such that the overall electron transfer in the C-N bond is in the direction of the nitrogen atom. In addition to this effect, the nitrogen is also expected to give rise to an electron shift from the other atoms covalently bonded to it, especially the NH hydrogen atom.

The C-N bond in amides provides an example of a common phenomenon in small molecules sometimes called "back transfer," meaning that electron shifts in different orbitals are in opposite directions. When an amide forms, the direction of shift of π-orbital electrons has to be from the lone pair of the nitrogen toward the carbonyl carbon atom (Poland & Scheraga, 1967; Wiberg & Breneman, 1992). The electronegativity of the nitrogen atom means

there is a compensating electron shift from C to N that occurs via the σ-orbitals.

Trigonal nitrogen atoms with partial negative charges, as in amides, are, in general, common in chemistry and biochemistry. They often occur in situations where the single p-orbital can overlap to form a π-bond with one or more adjacent groups. For example, the nitrogen atoms in the bases of nucleotides (Singh & Kollmann, 1984) are of this sort. In such nitrogens, the trigonal set of sp^2 electrons lies close to the nucleus, because of the high s-orbital character, and a p-orbital lies perpendicular to the others; this is thought (Laidig & Cameron, 1996) to satisfy the nitrogen atom's electronegativity better than sp^3 orbitals.

The stability of amides are now seen to result from two causes. One is the overlap of the p-orbital electrons from the nitrogen atom with the p-orbital of the carbonyl carbon. An alternative way of explaining this is the formation of a delocalized 3-center 4-π-electron system, as illustrated in Figure 2, but the calculations (Wiberg & Laidig, 1987) show that the major overlap is with the electron-deficient carbonyl carbon, rather than the oxygen, atom. The other amide-stabilizing effect is by means of favorable Coulombic interactions between covalently linked atoms. The carbonyl carbon and the nitrogen atoms are of opposite charge, and so attract each other. The polarization of this bond also leads to greater Coulombic stabilization of the carbonyl bond. This, in turn, stabilizes the C-N interaction. This purely electrostatic effect, added to the partial C-N π-orbital overlap, explains why the C-N bond is shorter in the planar form.

The *planarity* of the CONH group is caused mainly by the C-N π-orbital overlap, rather than by the scheme of Figure 1, and the arrangement is further stabilized by Coulombic interactions. Species b in Figure 1 with a C=N bond can still be regarded as a resonance form, although not a major one (Flegg & Harcourt, 1988), but the implication in Figure 1 that π-orbital overlap from N to C to O causes the oxygen to become negative is misleading because its charge is little affected by the degree of planarity of the group. The carbonyl group can, it is alleged (Wiberg & Breneman, 1992), be regarded as a spectator, rather than an active participant, in the rotation about the C-N bond; its passive function is to polarize the C=O bond to make the interaction possible. Species b also gives a false idea of the electron distribution because it takes no account of σ-orbitals.

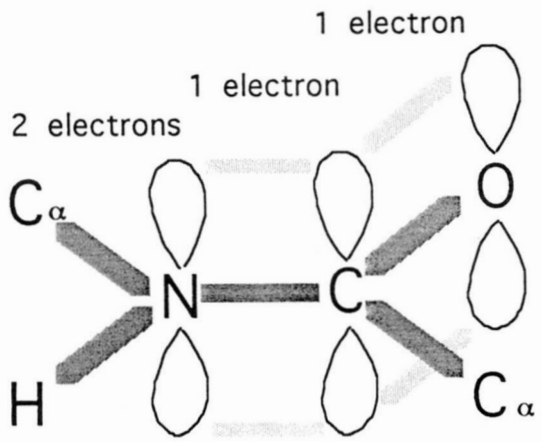

Fig. 2. In the planar form of the peptide bond, the six atoms shown are coplanar, with the p-orbitals perpendicular to the plane. The lighter shading shows the delocalized 3 center 4 electron π-orbital that is, in principle, formed by overlap of the lone pair electrons of the nitrogen p-orbital and the two electrons in p-orbitals of the carbonyl group. In the text, however, it is pointed out that, for the nitrogen, the major overlap is with the carbonyl carbon atom C, and that the electron shift from N to C (and the much smaller shift from N to C to O) via π-orbitals is at least counterbalanced by a movement of electron cloud in the opposite direction via the σ-orbitals.

Table 2. *Covalent bond orders for σ- and π-orbitals, bond lengths, and partial charges in formamide*

	Planar			90° Rotation		
A. Bond orders	π	σ	Total	π	σ	Total
C-O	0.46	0.67	1.13	0.57	0.68	1.25
C-N	0.23	0.66	0.88	0.05	0.84	0.89
C-H	0.00	0.90	0.90	0.00	0.92	0.92
NHa	0.03	0.75	0.78	nd	nd	0.83
NHb	0.03	0.76	0.79	nd	nd	0.83
N···O	0.13	0.15	0.29	0.06	0.16	0.22
B. Bond lengths		Å			Å	
C-O		1.19			1.18	
C-N		1.35			1.43	
C. Charges						
C		+1.98			+1.76	
O		−1.39			−1.34	
N		−1.48			−1.22	
H		+0.54			+0.59	

[a]Calculations (Wiberg & Breneman, 1992) were at the MP2/6-31G* level. On the left, the molecule is in a planar conformation and on the right, the C-N bond has been rotated by 90°.

Table 2A gives ab initio-calculated covalent bond orders (Cioslowski & Mixon, 1991; Wiberg & Breneman, 1992) for formamide in the planar conformation. At first sight, it is surprising, bearing in mind that the total bond order for the ethylene double bond is 1.96, that the value for the C=O bond is only 1.1. This suggests it has considerable ionic character. Charge density plots show that both π and σ components of the bond are polarized. Also, the total bond order for the C-N bond is only 0.88, and that for the σ-orbital is only 0.65; here the polarization of the σ-bond makes the nitrogen partly negative. Hence, the short C-O and N-C bond lengths are likely to be due to Coulombic attraction between the atoms as well as to the π-orbitals.

Also in Table 2A are given the bond order data for a formamide conformer, where the C-N bond has been made to rotate by 90°. The nitrogen is pyramidalized, the C-N bond length has increased, and the C-N π-orbital bond order is very low. This is as expected because of the nonplanarity. The other C-N (σ) and the C-O (σ and π) bond orders are somewhat increased. This is consistent with their having lost some of the ionic character of the planar form (except that the net charge on the oxygen is little changed), because the carbonyl carbon and nitrogen atoms are behaving more independently.

As a simple way to portray peptides, it has been suggested (Poland & Scheraga, 1967; Flegg & Harcourt, 1988; Wiberg & Rablen, 1993, 1995) that the three resonance forms in Figure 3 are more appropriate than the two in Figure 1. If only the π-orbitals are being portrayed, this gives a fair idea of the situation. However, it does not show the partial charge on the nitrogen atom correctly because it fails to take account of the polarization in the C-N σ-bond, which is opposite to that of the π-bond.

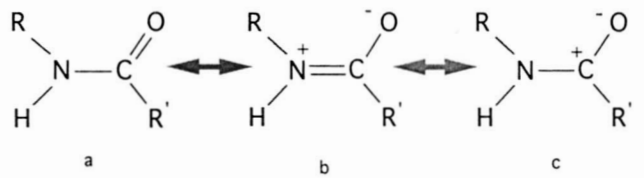

Fig. 3. π-Orbitals of the peptide bond are better represented by three resonance forms, rather than the two in Figure 1. This representation still gives a false idea of the overall electron distribution because it takes no account of back transfer via the σ-orbitals.

Pictures (Laidig & Bader, 1991; Price & Stone, 1992) representing the distribution of electrostatic energy derived from quantum mechanical calculations show that different parts of the nitrogen atom have different amounts of charge density, so it could be said to be difficult to represent the nitrogen as having any single charge. However, the results show that the most negative part is at the surface immediately opposite to the NH hydrogen atom, so the N-H dipole is still evident with this approach.

Effect of hydrogen bonding on amide structure: Comparison (Kitano & Kuchitsu, 1974; Ottersen, 1975; Jeffrey et al., 1980; Jeffrey & Saenger, 1991) of gas phase and crystal structure bond lengths in acetamide reveals the effect of hydrogen bonding on the structure, because it is hydrogen bonded in the crystal. Going from gas phase to crystal, there is a small decrease (0.044 Å) in the N-C distance and a smaller increase (0.027 Å) in the C-O distance. This can be interpreted (Jeffrey & Saenger, 1991) in terms of the scheme of Figure 1; however, the quantum mechanical insights in the section above suggest an improved interpretation. It can be presumed that, when there is hydrogen bonding, the partial charges on the carbonyl oxygen and the NH hydrogen atoms become exaggerated; because of some extra electron transfer from C to O and from H to N, the charges on the carbonyl carbon and the nitrogen atoms likewise become accentuated. In other words, the bonds become more polarized. Given bond orders like those in Table 2, a lengthening of the C-O bond is to be expected because the π-electron overlap is less; on the other hand, the C-N distance is expected to decrease because of the combined effects of an increased C-N π-electron overlap plus a higher electrostatic C-N attraction. Hence, hydrogen bonding confers extra rigidity to the peptide bond.

Relevance to aspects of proteins: This insight into the nature of peptide bonds is relevant to several aspects of protein structure. It means that main-chain–main-chain N-H···O=C hydrogen bonds can be regarded as two dipoles arranged head to tail and pointing in the same direction. Much of the favorable interaction of such hydrogen bonds, and also their directionality, can be explained by the electrostatic attraction between the dipoles.

This is of particular relevance to α-helices, as shown in Figure 4. Their central part consists of linked N-H···O=C-N-H···O=C groups with the N-H and O=C units parallel to the helix axis and their dipoles pointing in the same directions. Each turn of polypeptide strand can be regarded as having a positive side and a negative side. More sophisticated electrostatic models of polypeptides (Price & Stone, 1992) are in broad agreement with this. Energy calculations (Maccallum et al., 1995) on proteins of

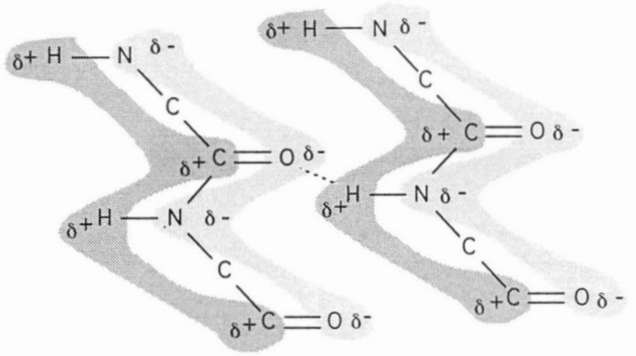

Fig. 4. Successive turns of an α-helix have a positive side and a negative side, which are shaded differently. Apart from the hydrogen bonding, they are also held together by purely electrostatic interactions (Maccallum et al., 1995).

known 3D structure show that, as well as the well-known hydrogen bonding, there are substantial attractive non-hydrogen bonded electrostatic interactions between adjacent turns of helix; the sum of their energies is nearly as high (in a negative sense, negative energies being favorable) as that due to the hydrogen bonds.

At the ends of helices, a turn of polypeptide is invariably left that lacks a hydrogen bond or electrostatic partner; at the C-terminus, it is the negatively charged side that is exposed and, at the N-terminus, it is the positively charged side. This is why the N-termini of α-helices often function as binding sites for inorganic anions (Hol et al., 1978; Chakrabarti, 1993; He & Quiocho, 1993) or for the phosphates of nucleotides (Schulz, 1992; Sayle & Milner-White, 1995) or are occupied by carboxylate side chains (Harper & Rose, 1993; Doig et al., 1997). Earlier work (Hol et al., 1978) ascribed the binding of anions at helical N-termini to an interaction with the macrodipole of the entire helix. It seems more realistic, however, to regard them (He & Quiocho, 1993) as interacting with the local dipoles of the CONH groups of the first turn or two of the helix.

α-Helices are thought to be cooperative (Hol et al., 1978; Jeffrey & Saenger, 1991) in the sense that the formation of subsequent hydrogen bonds (plus associated electrostic interactions) is more favorable than the formation of an α-helix with just one hydrogen bond. This is why most naturally occurring helices have a tendency to be long rather than short, i.e., classic α-helices comprising just two or three hydrogen bonds are comparatively uncommon. There are two effects. One is the obvious one that, once one hydrogen bond has formed, it is easier for subsequent bonds to form by a zipper effect. The other is that there may be an extra polarization of the bonds in the CONH group, causing an exaggeration of the values of the partial charges, rather as described when discussing the effect of hydrogen bonding in the previous section, but more so, due to the effect of having linked N-H···O=C-N-H···O=C groups (and the associated electrostatic interactions).

There have been reports (Kearley et al., 1994) suggesting that, in α-helices, the hydrogen bonding cooperativity is so effective in polarizing the N-H groups of the peptide bond that the protons are able to dissociate; if so, an ionic ($N^{\delta-}\cdots H^{+}\cdots O^{\delta-}$), rather than a hydrogen bonded (N-H···O) representation would be appropriate for the main chain of the α-helix. This is an appealing concept, including the possibilities of proton tunneling and the idea that a proton might be transferred rapidly the complete length of a helix.

However, it has to be regarded as controversial because there is good evidence (Perrin, 1994) that, although NH···CO hydrogen bonding may be stronger in an α-helix than on its own, proton dissociation is simply not favored chemically.

Conclusions: I have pointed out that, in the CONH groups of polypeptides, in spite of the ease of drawing a resonance species with a positive charge on the nitrogen, this atom is not thought to have a net partial positive charge. The precise current of charge is uncertain, but the evidence, from quantum mechanics calculations, is strongly that it is negative. This arises, in part, because the electronegativity of the nitrogen attracts electrons along the C-N σ-bond, in the opposite direction to the electron shift that occurs when the lone pair on the nitrogen is shared with the carbonyl carbon atom via π-bonding.

The low values for the bond orders derived by quantum mechanics calculations (Wiberg & Breneman, 1992) for the N-C and C-O bonds of amides, combined with the high opposite partial charges on the adjacent atoms, mean that the covalent bonding in amides has considerable ionic character. Atoms are held together to a significant extent by Coulombic interactions, as well as by electron orbital overlap. Hydrogen bonding is likely to increase the values of the partial positive and negative charges somewhat and thus accentuate the electrostatic components of the covalent N-C and C-O bonding.

Rather than represent amides by the usual two resonance forms normally depicted, it is more realistic to include a third form with a positive charge on the carbonyl carbon and a negative charge on the oxygen atom. This gives a fair picture of the π-orbitals, but still fails to show the shift in electron cloud in the σ-orbitals.

Acknowledgments: I thank Brian Webster, David Morris, John Carnduff, Paul Mallinson, Andy Karplus, and David Rycroft for help.

References

Abeles RH, Frey PA, Jencks W. 1992. *Biochemistry*. Boston: Jones & Bartlett.

Bailey PD. 1990. *An introduction to peptide chemistry*. New York: Wiley.

Branden CI, Tooze J. 1991. *Introduction to protein structure*. New York: Garland.

Chakrabarti P. 1993. Anion binding sites in protein structures. *J Mol Biol 234*:463–482.

Chang R. 1996. *Essential chemistry*. New York: McGraw-Hill.

Cioslowski J, Mixon ST. 1991. Covalent bond orders in the topological theory of molecules. *J Am Chem Soc 113*:4142–4156.

Cornell JW, Cieplak P, Bayly CI, Gould IR, Merz KM Jr, Ferguson DM, Spellmeyer DC, Fox T, Caldwell JW, Kollman PA. 1995. A second generation force field for the simulation of proteins, nucleic acids and organic molecules. *J Am Chem Soc 117*:5179–5197.

Creighton TE. 1993. *Proteins: Structures and molecular properties, 2nd ed.* New York: Freeman.

Darnell JE, Lodish H, Baltimore D. 1986. *Molecular cell biology*. New York: Scientific American Books.

Doig AJ, Macarthur MW, Stapley BJ, Thornton JM. 1997. Structures of N-termini of helices in proteins. *Protein Sci 6*:147–155.

Dunitz JD, Winkler FK. 1983. Amide group deformation in medium ring lactams. *Acta Crystallogr B 31*:251–263.

Ege S. 1990. *Organic chemistry, 2nd ed.* Lexington, MA: D.C. Heath.

Elliott WH, Elliott DC. 1997. *Biochemistry and molecular biology*. Oxford: OUP.

Fersht AR. 1971. Acyl transfer reactions of amides and esters. Concerning the N-protonation of amides and amide-imidate equilibria. *J Am Chem Soc 93*:3504–3521.

Fersht AR. 1985. *Enzyme structure and mechanism, 2nd ed.* New York: Freeman.

Fessenden RJ, Fessenden JS. 1986. *Organic chemistry, 3rd ed.* Brooks/Cole.

Flegg RH, Harcourt RD. 1988. Ab initio valence bond studies of formamide. *J Mol Struct (Theochem) 164*:67–81.

Fox MA, Whitesell JK. 1997. *Organic chemistry, 2nd ed.* Boston: Jones & Bartlett.

Gatti C, Bianchi R, Destro R, Merati F. 1992. Experimental vs. theoretical topological properties of charge density distributions. An application to the L-alanine molecule studied by X-ray diffraction at 23 °K. *J Mol Struct (Theochem) 255*:409–453.

Gilli G, Bertolasi V, Belluci F, Ferretti V. 1986. Stereochemistry of the $R_1(X=)C(sp^2)$-$N(sp^3)R_2R_3$ fragment. Mapping of the *cis–trans* isomerisation path by rotation around the C-N bond from crystallographic structure data. *J Am Chem Soc 108*:2420–2424.

Glusker JP, Lewis M, Rossi M. 1991. *Crystal structure analysis for chemists and biologists*. New York: VCH Publishers.

Hamaguchi K. 1994. *The protein molecule*. Japan Scientific Societies Press/ New York: Springer-Verlag.

Harper ET, Rose GD. 1993. Helix stop signals in proteins and peptides: The capping box. *Biochemistry 32*:7606–7609.

He JJ, Quiocho FA. 1993. Dominant role of local dipoles in stabilising uncompensated charges on a sulphate sequestered in a periplasmic active transport protein. *Protein Sci 2*:1643–1647.

Hol WGJ, van Duijnen PT, Berendsen HJC. 1978. The α-helix dipole and the properties of proteins. *Nature 273*:443–446.

Homer RB, Johnson CD. 1970. The acid/base and complexing properties of amides. In: Zabicky J, ed. *The chemistry of amides*. New York: Wiley. pp 187–243.

Jeffrey GA, Ruble JR, McMullan RK, DeFrees DJ, Binkley JS, Pople JA. 1980. Neutron diffraction study at 23 °K and ab initio molecular orbital studies of acetamide. *Acta Crystallogr B 36*:2292–2299.

Jeffrey GA, Saenger W. 1991. *Hydrogen bonding in biological structures*. New York: Springer-Verlag.

Kabsch W, Sander C. 1983. Dictionary of protein secondary structure. *Biopolymers 22*:2577–2637.

Karplus PA. 1996. Experimentally observed conformation-dependent geometry and hidden strain in proteins. *Protein Sci 5*:1406–1420.

Kearley GJ, Fillaux F, Baron MH, Bennington S, Tomkinson J. 1994. A new look at proton transfer dynamics along the hydrogen bonds in amides and peptides. *Science 264*:1285–1289.

Kitano M, Kuchitsu K. 1974. Molecular structure of formamide as studied by gas electron diffraction. *Bull Chem Soc Japan 47*:67–72.

Laidig KE, Bader RFW. 1991. Distorted amides: Correlation of their enhanced solvolysis with local charge depletions at the carbonyl carbon. *J Am Chem Soc 113*:6312–6313.

Laidig KE, Cameron LM. 1996. Barrier to rotation in thioformamide: Implications for amide resonance. *J Am Chem Soc 118*:1737–1742.

Lehninger AL, Nelson DL, Cox MM. 1992. *Principles of biochemistry, 2nd ed.* New York: Worth.

Maccallum PH, Poet R, Milner-White EJ. 1995. Coulombic interactions between partially charged main chain atoms not hydrogen bonded to each other influence the conformations of α-helices and β-sheet. *J Mol Biol 248*:361–373.

Mathews CK, van Holde KE. 1996. *Biochemistry, 2nd ed.* Menlo Park, CA: Benjamin-Cummings.

McMurray J. 1991. *Organic chemistry, 4th ed.* Brooks/Cole.

Murray RK, Granner DK, Mayes PA, Rodwell VW. 1996. *Harper's biochemistry, 24th ed.* Stanford, CT: Prentice-Hall Intl.

Ottersen T. 1975. On the structure of the peptide linkage. The structures of formamide, acetamide and N-methyl formamide at −165 °C. *Acta Chem Scand A 29*:939–944.

Palmer T. 1995. *Understanding enzymes, 4th ed.* London, UK: Prentice-Hall.

Perrin CL. 1994. Symmetries of hydrogen bonds in solution. *Science 266*:1665–1668.

Poland D, Scheraga HA. 1967. Energy parameters in polypeptides: Charge distributions and the hydrogen bond. *Biochemistry 6*:3791–3800.

Price SL, Stone AJ. 1992. Electrostatic models for polypeptides: Can we assume transferrability? *J Chem Soc Faraday Trans 88*:1755–1763.

Reynolds CA, Essex JW, Richards WG. 1992. Atomic charges for variable molecular conformations. *J Am Chem Soc 114*:9075–9079.

Rogers NK. 1986. The modelling of electrostatic interactions in the function of globular proteins. *Prog Biophys Mol Biol 48*:37–66

Sayle RA, Milner-White EJ. 1995. RASMOL: Biomolecular graphics for all. *Trends Biochem Sci 20*:373–375

Schulz GE. 1992. Binding of nucleotides by proteins. *Curr Opin Struct Biol 2*:61–67.

Singh UC, Kollmann PA. 1984. An approach to computing electrostatic charges for molecules. *J Comp Chem 5*:129–145.

Solomons TWG. 1994. *Organic chemistry, 3rd ed.* New York: Wiley.

Stryer L. 1995. *Biochemistry, 4th ed.* New York: Freeman.

Voet D, Voet JG. 1990. *Biochemistry*. New York: Wiley.
Webster B. 1990. *Chemical bonding theory*. Oxford: Blackwell.
Weiner SJ, Kollman PA, Case D, Singh UL, Chio C, Alagona G, Profeta PS, Weiner P. 1984. A new force field for molecular mechanical simulation of nucleic acids and proteins. *J Am Chem Soc 106*:765–784.
Wiberg KB, Breneman CM. 1992. Resonance interactions in acyclic systems. 3. Formamide internal rotation revisited. Charge and energy redistribution along the C-N bond rotational pathway. *J Am Chem Soc 114*:831–840.
Wiberg KB, Glaser R. 1992. Resonance interactions in acyclic systems. 4. Stereochemistry energetics and electron distributions for 3 center 4 electron systems A=B-C. *J Am Chem Soc 114*:841–850.
Wiberg KB, Hadad CM, Rablen PR, Cioslowski J. 1992. Substituent effects. 4. Nature of substituent effects at carbonyl groups. *J Am Chem Soc 114*:8644–8654.
Wiberg KD, Laidig KE. 1987. Barriers to rotation adjacent to double bonds. 3. The CO barrier in formic acid, methyl formate and methyl acetate. The origins of ester and amide "resonance." *J Am Chem Soc 109*:5935–5943.
Wiberg KB, Rablen PR. 1993. Substituent effects. 5. Vinyl and ethynyl derivatives. An examination of the interaction of amino and hydroxy groups with C-C double and triple bonds. *J Am Chem Soc 114*:8644–8654.
Wiberg KB, Rablen PR. 1995. Why does thioformamide have a larger rotational barrier than formamide? *J Am Chem Soc 117*:2201–2209.
Zubay G. 1993. *Biochemistry, 3rd ed.* Oxford: WCB.
Zubay G, Parson WW, Vance DE. 1995. *Principles of biochemistry*. Oxford: WCB.