Linus Pauling and the planar peptide bond

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Every first year biochemistry student learns about the planarity of the peptide bond. Planarity, the result of ~40% N–C′ double bond character arising from two dominant resonance structures, allows for a great simplification in the understanding of protein structures. Of the three repeating protein backbone dihedral angles, only ϕ and ψ need to be considered when assigning secondary structure, because the peptide bond dihedral angle ω is generally considered fixed at 180°. Linus Pauling's prediction of the α-helix, one of the greatest achievements in structural biology, was made by assuming (i) that the peptide bond is planar, (ii) that all amino acid residues are equivalent with respect to backbone conformations and (iii) that each amide proton is hydrogen bonded to an oxygen atom of another residue with an N–O distance of 2.72 Å (ref. 1). Similar criteria allowed Pauling to predict several other protein secondary structural elements including the γ-helix and parallel and antiparallel β-sheets, accomplishments that resulted in his 1954 Nobel Prize in Chemistry.

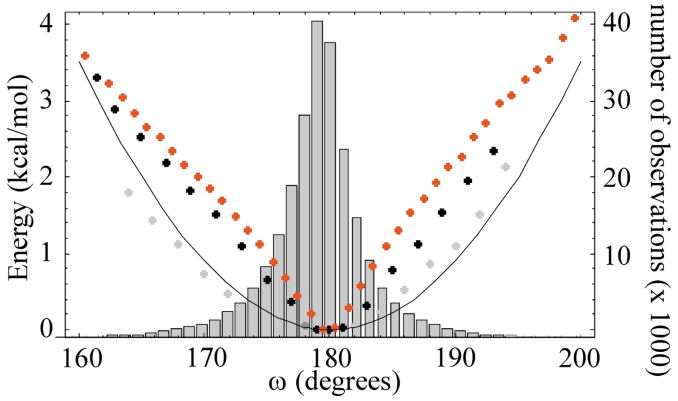

Fig. 1 Peptide bond rotational energies and angular frequency distribution. The black and gray points are from ref. 7 and were derived from Maxwell-Boltzmann relations for proteins and peptides, respectively. The histogram represents the angular frequency distribution of 237,807 values of ω from coiled regions of 3,938 proteins in the current release of the protein data base (January, 2001) with resolution ≤ 2.0 Å and R-factor ≤ 20%. The red points are energies derived from Maxwell-Boltzmann relations of the current values of ω, as described in ref. 7. The line is the function $A \sin^2\omega$ for A = 30 kcal mol^{-1}, as derived by Corey and Pauling in ref. 8.

In 1968, Ramachandran recognized the need for nonplanar peptide bonds in cyclic peptides[2]. In later work, the same group argued that discrepancies in calculated values of $^3J_{HN-H\alpha}$ scalar coupling constants in peptides with bulky side chains showed the need to allow for nonplanar peptide bonds in both cyclic and linear peptides[3]. They also noted that most of calculations up to that time used "the standard, completely planar, *trans* peptide unit of dimensions given by Pauling and Corey[4] as early as 1951"[3]. Much later and with relatively high level Hartree-Fock extended basis set *ab initio* calculations, Pople's group showed that the peptide bond in the gas phase has significant flexibility and rotates as much as 40° with little energetic cost[5], a result subsequently verified by Edison and coworkers[6]. Simple geometric drawings will show that deviations of this size result from relatively small changes in interatomic distances.

In a statistical survey of peptide and protein databases, Thornton's group convincingly showed that experimentally derived peptide and protein structures have significant deviations from planar peptide bonds[7]. Using data from 492 peptide bonds in the Cambridge Structural Database and 45,851 *trans* peptide bonds from 187 different protein structures in the Brookhaven Protein Data Bank (now the Research Collaboratory for Structural Bioinformatics Protein Data Bank), they were able to estimate the energies of peptide bond rotation using Maxwell-Boltzmann statistics (recreated in Fig. 1)[7]. Fig. 1 also provides updated values to Thornton's survey that are in qualitative agreement with the results from 1996. Interestingly, in both the original study and updated values, there is a small but significant tendency toward angles <180°. Thus, both theory and experiment on both small peptides and proteins suggest that Linus Pauling's famous planar peptide bond is overly simplistic.

Could Linus Pauling, one of the world's greatest chemists, have failed to understand that some flexibility is clearly a real (and quite likely important) property of the peptide bond? Several introductory and summary statements made by Pauling show the importance of peptide bond planarity: "The normal coplanarity of the atoms of [the peptide bond] is the result of resonance which gives rise to partial double bond character of the N–C′ peptide bond. Rotation about this bond is, in general, severely restricted." (abstract of ref. 8); "The normal planarity of the amide group is established on both experimental and theoretical grounds as a sound structural principle. A structure in which the atoms of the amide group are not approximately coplanar should be regarded with scepticism until its relatively unstable configuration has been adequately confirmed." (conclusion of ref. 8); "The N–C bond has about 40 percent double-bond character (bond length 1.32 Å). The group is planar, and it has been found to have the trans configuration in all substances studied except the cyclic peptides (diketopiperazine)." (page 498 of ref. 9).

Pauling, like any great chemist, clearly saw the importance of generalization. He was a master at reducing complicated problems to their essence and stressing what he knew to be the most important points, which are emphasized as the major conclusions of his work. A more thorough reading, however, shows that he was keenly aware of the flexibility of the peptide bond: "About 40% double-bond character appears to be associated with the C′–N peptide bond. We may therefore estimate the strain energy involved in rotation around this bond. If the planes of the two ends of the amide group form a dihedral angle δ, and if A is the amide resonance energy for the planar configuration, the strain energy may be taken equal to $A \sin^2\delta$. A reasonable value for A is about 30 kcal/mole. From this we can calculate strain energies of about

0.9 kcal/mole for 10 degree distortion of the amide group, 3.5 kcal/mole for 20 degree distortion, and so on." (page 14 of ref. 8).

A plot of Corey and Pauling's 1953 energy function for rotation of the peptide bond is shown alongside the 1996 and most recent estimates based upon the statistical survey of the peptide and protein data bases. Nearly 50 years ago and without the benefit of a single high resolution protein structure or supercomputer, Pauling got it right.

Acknowledgment
I thank H. Weissig of the San Diego Supercomputer Center and Protein Data Bank for providing the dihedral angles used in Fig. 1. D. Purich, R. McKenna, M. Agbandje-McKenna, and members of my laboratory provided helpful and stimulating discussions.

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Received 7 December, 2000; accepted 31 January, 2001.

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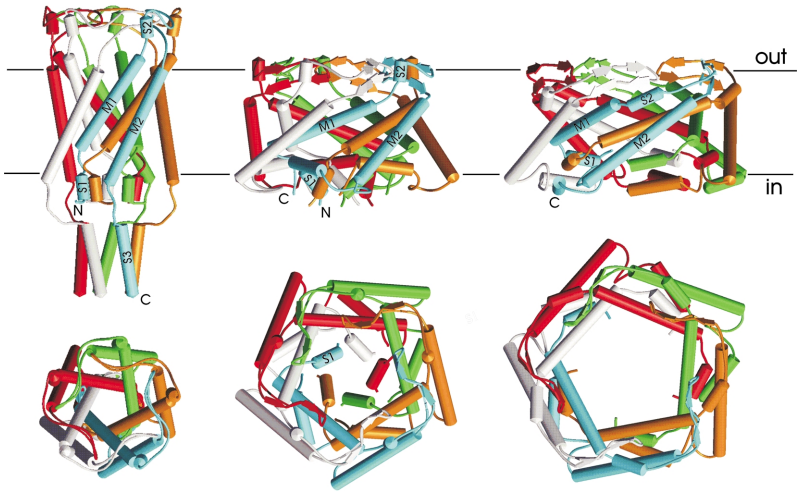
Leak proof channel

Tension can be a killer. This is especially true when pressure builds up in single cell organisms such as bacteria. To deal with rapid changes in osmotic pressure, bacteria have evolved safety valves in the form of mechanosensitive channels. In many organisms such channels convert mechanical strain induced by sound, touch, gravity or pressure into an electrochemical response. When tension within the cell increases, these channels open to release pressure and osmolytes quickly and keep the bacterium alive. While this channel is a lifesaver in times of stress, at other times it needs to be completely closed to maintain the electrical integrity of the membrane. A leaky channel would eventually kill the bacterium.



To gain an understanding of the gating mechanism of such mechanosensitive channels, Sukharev and coworkers (*Nature* 409, 720–724 (2001)) developed a number of structural models based on the previously solved crystal structure of the mechanosensitive channel of large conductance, MscL, from *Mycobacterium tuberculosis* (the closed structure was solved to 3.5 Å resolution) and previous patch clamp experiments on the *Escherichia coli* MscL channel.

Features of the crystal structure and the energetic parameters of the bacterial channel were incorporated into a series of molecular models. The crystal structure showed that the channel is composed of five identical subunits (each one is a different color). Within each subunit, helical segments M1, M2 and S3 (shown as cylinders) were observed. The structure suggested that the M1 helices formed the gate.

Thermodynamic analyses of the MscL channel predicted that the channel opens through pre-expanded intermediate states. The closed conformation (left) expands to two-thirds of its open size to form the so-called closed-expanded conformation (middle) before it actually opens (right). Channel opening could occur by tilting of the M1 helices away from the center of the pore like the iris of an eye. Moreover, the ability of the channel to go from the closed-expanded conformation to the open conformation without a substantial change in the overall size of the channel suggested the presence of a second gate. Given that S2 is poorly conserved and S3 is dispensable, the best candidate for a second gate was S1, which was disordered in the crystal structure. Sukharev and coworkers modeled the S1 segments as short helices that block the pore when the channel is in the closed conformation.

To test their model for the role of S1, they substituted cysteines for specific S1 residues and asked whether these residues were close together in neighboring subunits in the closed conformation and whether crosslinking of S1 prevented channel opening. They found that S1 segments form a bundle when the channel is closed and crosslinking between S1 segments prevents opening. They also found that S1 segments crosslink with an outer helix (M2) when the channel is open, and this impedes channel closing.

Thus, the MscL channel appears to have two gates in series to ensure that there is no leakage when the channel is closed. The cytoplasmic (S1) gate 'feels' the tension only when the barrel is critically distorted and the flexible linkers connecting S1 to M1 are in the extended conformation. In this way, these channels serve as 'emergency release valves' when the going gets tough and remain absolutely closed when life is stress free.

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