two cGMP-dependent conductances. Usually, the presence of cone photoreceptor material in a bovine retina preparation is thought to be minimal, but is it possible that one channel is from cones and the other from rods? Cook *et al.* find that L-*cis*-diltiazem perfused into the rod cell does not block the light-sensitive conductance and suggest that their molecule has properties of this channel form. There may, however, be other reasons, such as hydrolysis or competition, why the perfusion does not work. There is no other compelling physiological evidence that more than one channel exists. If there are two in one cell type, why?

Both groups should be able to resolve whether the polypeptides are related by the combined use of discriminating antibodies, peptide mapping and sequencing. Then we can look forward to learning just how this type of channel is controlled. Is it modulated by phosphorylation or by interaction with other regulatory proteins? Cyclic-nucleotide-gated conductances are now being identified in other sensory systems and may well reside in other neurons[13]. With the biochemical questions dispelled, this channel promises to serve as an informative molecular model for cyclic-nucleotide-gated ion channels. □

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Protein structure

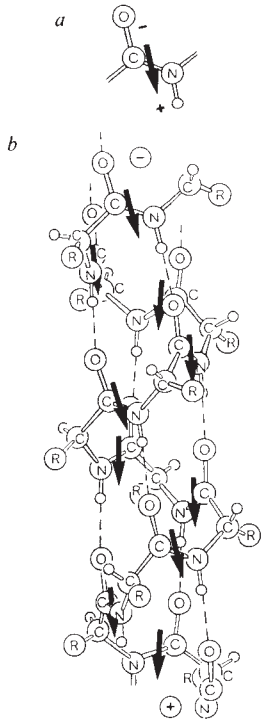
Stability of alpha-helices

Thomas E. Creighton

PROTEINS normally exist in well-defined three-dimensional conformations, in contrast to the usual disordered state of other polymers. The physical basis of the stabilities of these folded conformations is only partially understood, even though they are essential for most biological phenomena. Best understood seemed to be the relatively simple isolated α-helix, but recent work by Baldwin and co-workers, reported on page 563 of this issue[1], identifies one factor that has been overlooked and indicates that others remain to be discovered.

The formation of α-helices in disordered polypeptides is a classical nucleation event, usually treated by the Zimm–Bragg theory[2]. Formation of the first segment of the helix from the disordered polypeptide is rate-limiting and energetically unfavourable, with an equilibrium constant, σ, typically of the order of 10^{-4}. Addition of amino-acid residues to either end of the helix is very rapid and occurs with an equilibrium constant, s, close to unity. The unfavourable nature of the nucleation event probably results from the need to fix, simultaneously and independently, at least four contiguous residues in the helical conformation, to give at least one complete helical turn and the first hydrogen bond; in contrast, growth of the helix occurs by addition of individual residues (see figure).

Different types of amino-acid residues have different values of s, but the Zimm–Bragg theory assumes that this single value pertains to both ends of the helix, and is independent of the neighbouring sequence. Using this theory, and measuring the effect of a few 'guest' amino-acid residues on the helicity of a 'host' polypeptide, Scheraga *et al.* have measured the values of σ and s for 18 of the 20 amino-acid residues. The values of s at 25 °C range from 0.6 for Gly to 1.32 for nonionized Glu[3]. The Zimm–Bragg theory predicts that only polypeptides of amino acids with $s \geq 1$ should form stable helices, and then only with long polypeptides. With $\sigma = 10^{-4}$, for example, a long homopolypeptide with $s = 1$ would consist of stretches of helix interspersed with disordered segments, each averaging 100 residues in length. Short peptides of less than 20 residues are not expected to form stable helices; for example, even with the maximum observed value of $s = 1.3$, a 13-residue peptide would be expected to be helical only 8 per cent of the time.



a, Dipole of a single peptide bond. *b*, Individual peptide-bond dipoles within an α-helix, showing how they are aligned nearly head-to-tail.

The model explains most observations with homopolypeptides and the usual instability of helices in isolated peptides, but there are some exceptions. Ooi and co-workers[4], for example, observed greater helical content in a segment of Ala residues when in the sequence Glu_{20}–Ala_{20}–Phe than in Ala_{20}–Glu_{20}–Phe, suggesting that the value of s is dependent on position in the polypeptide. Also, some small peptides have much greater than expected helical contents. Brown and Klee[5], for example, observed significant amounts of helix in the C-peptide, although only at low temperatures. (C- and S-peptides are cleaved from ribonuclease A by cyanogen bromide and subtilisin, respectively.) The C-peptide, consisting of the 13 amino-terminal residues of ribonuclease A, contains no residues with $s > 1.09$; residues 3–13 exist in a stable helical conformation in folded ribonuclease A. Using the C-peptide, the S-peptide (residues 1–20), and synthetic homologues, these observations are now confirmed and extended by Baldwin and co-workers[1] and by Rico *et al.*[6]. These authors observe helical contents of up to about 50 per cent and localize the helix to only the amino-terminal portion of the S-peptide[7], as in folded ribonuclease. The variation of helicity with pH and with salt concentration indicates that electrostatic interactions are partially responsible, but the initial suggestion of a particular stabilizing salt-bridge between side chains was disproved. Nevertheless, the effects of charged residues are dependent on their position in the peptide. The helical content is increased by positively charged groups near the carboxyl end and by negatively charged groups at the amino end, and is decreased when they are at the other ends. There is a similar pattern in folded proteins, where there is a tendency for charged residues to occur at that end of the helix at which they stabilize it[8].

The explanation offered for these observations is that the charged groups interact with the dipole of the helix, as had been suggested by Blagdon and Goodman[8]. The helix dipole arises from the substantial dipole of the peptide bond (about 3.5 Debye units, or 1.2×10^{-29} Cm),

several of which are aligned nearly head-to-tail in the α-helix. Consequently, each end of the helix may have an effective net charge of about half an electron, being positive at the amino end. Hol has recently proposed[9] roles for the helix dipole in protein structure and function, particularly for ligand binding. Earlier, Brant and Flory[10] stressed the importance of the helix dipole for protein conformation and helix stability, pointing out that energetically unfavourable parallel alignments of the peptide-bond dipoles would inhibit nucleation of the helix, whereas its further growth would be aided by head-to-tail alignment of the dipoles. The new observations[1,5] indicate that the helical state is stabilized by interactions of oppositely charged groups with either end of the helix dipole, and that it is destabilized by unfavourable interactions.

These electrostatic interactions, however, have only a relatively small effect; a single charge interaction has been shown to alter the helical content by at most threefold, and usually by only twofold. Even without such stabilizing interactions, the S-peptide α-helix is about 30-fold more stable than would have been predicted, so the major interactions that stabilize this particular helix remain unknown.

Most important, systematic studies of helix stability in small peptides are now feasible, and Scheraga and co-workers[3] are using the experimental data to develop an extension of the Zimm–Bragg theory that will take into account interactions of each side chain with the helix dipole and with other side chains. Predicting the helical propensities of different amino-acid sequences should be greatly improved, certainly when in an isolated peptide and possibly within folded proteins. It seems clear, however, that helical conformations in folded proteins are ultimately determined by tertiary interactions with the rest of the protein[11]. Nevertheless, Mitchinson and Baldwin[12] were able to produce semisynthetic forms of ribonuclease S with increased thermostabilities by combining their more helical S-peptide homologues with the normal S-protein. Stabilizing proteins by stabilizing their α-helices, and perhaps other elements of secondary structure, should become possible whenever the required covalent structure changes do not affect the internal packing or other stabilizing interactions of the folded protein.

Because α-helices are favourite candidates for nucleation sites in folding proteins, the greater than expected stability of some may tend to increase this popularity. But in my opinion, the observed kinetics of protein unfolding and refolding are inconsistent with a nucleation event being the overall rate-limiting step, like that in helix formation. Partially folded intermediates undoubtedly are important before the rate-limiting step, and helices may play a role in stabilizing them. Yet the surprisingly stable S-peptide helix I have discussed here is reported to appear only at the same time as most of the ribonuclease tertiary structure, not in the observed initial steps of folding[13]. Perhaps too much emphasis has been placed on elements of secondary structure. □

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Variations of AIDS virus relatives

ALTHOUGH one of the hallmarks of HIV-1, the human immunodeficiency virus type 1 that causes AIDS, is the considerable variability between independent isolates, on page 610 of this issue James Mullins and his colleagues report[1] that a remarkable invariability seems to characterize isolates of a related group of viruses with a somewhat nebulous relationship to AIDS. The alternative interpretation considered by the authors — and the subject of rumours for some months — is that the viruses are not all independent isolates but rather the result of laboratory contamination.

The group of viruses studied by Mullins's team comprises three monkey viruses and one human virus. The latter is HTLV-4, the human T-cell lymphotropic virus type 4, which was first isolated from healthy Sengalese and has since been shown by serological means to be present elsewhere in western Africa, including Ivory Coast, where it is widely prevalent[2]. The three monkey viruses are STLV-3_{mac}, STLV-3_{agm} and STLV-3_{smm}, the simian T-cell lymphotropic viruses type 3 isolated from the rhesus macaque, the African green monkey and the sooty mangabey monkey, respectively. Only the first of these seems to cause immunodeficiency in its host.

In a paper published at the tail end of last year Mullins, M. Essex and colleagues described[3] the molecular cloning of STLV-3_{agm} from an infected cell line and a consensus restriction-enzyme map of the predominant form of the virus in the cells. Mullins and his collaborators have now been able to compare this map with maps of HTLV-4 (which they cloned from an infected cell line provided by the Essex laboratory), STLV-3_{mac} (provided by R. Desrosiers) and additional isolates of STLV-3_{agm} (provided by Essex). What is remarkable about these maps is that they are all much the same, although a few variations are detected both between the different viruses and between different isolates of the same virus type.

The similarity is all the more remarkable when HIV-2, the virus isolated by Luc Montagnier and his colleagues from AIDS patients in western Africa, is taken into account. According to Montagnier's group[4], HIV-2 is prevalent in the same areas as HTLV-4 and the two are clearly closely related. And yet whereas the restriction maps of the three isolates of HTLV-4 reported in this issue are almost identical to each other, that of an isolate of HIV-2 is very different and there is considerable variation among HIV-2 isolates[4].

Mullins and colleagues suggest two interpretations of these data. The first is that "STLV-3_{agm}, STLV-3_{mac} and HTLV-4 may represent a remarkably stable virus capable of transmission between several species". In favour of that interpretation, they note that two of the STLV-3_{mac} viruses were isolated three years apart and yet have identical restriction maps. They also note that HTLV-1 and STLV-1 are very similar in structure and each can infect other species. The alternative interpretation put forward is that some of the viruses "do not correspond to independent virus isolates but originate from other virus-producing cell cultures maintained in the same laboratories"[1]. If that is the case, the most likely possibility is that STLV-3_{mac} is masquerading as STLV-3_{agm} and HTLV-4.

If contamination can be excluded — and that will probably require the isolation under stringent conditions of further examples of the viruses — there will be several very interesting questions to answer. Why is there such stability in one group of viruses but such variability in others? Does it reflect a shift from a non-pathogenic virus, HTLV-4, to a pathogenic form, HIV-2? And, if it does, what differences in sequence account for the shift? Whatever the real identity of the cloned HTLV-4, sight should not be lost of the fact that 1–5 per cent of several populations of west Africans are infected with HIV-2/HTLV-4 and are consequently at some risk of developing AIDS. □

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