society would seem to have been fundamentally different from that of modern man after 30–35 kyr ago. On the other hand, the rates of Neanderthal evolution between the end of the last interglacial and 35 kyr ago may have to be halved. The interval between the latest classical Neanderthals and the earliest *Homo sapiens sapiens* in Europe may have been sufficiently long for the former to have evolved into the latter. Unfortunately, most Neanderthal remains are still very poorly dated, and will remain so until the incoming generation of ^{14}C techniques provide reliable age determinations back to 100 kyr ago[15]. Nevertheless, many of these finds may turn out to be much older than previously thought, and the gaps between them much longer. Indeed, the important Mousterian site of Tata in Hungary has recently been redated[16] from 55 to 110 kyr BP. Whether the archaeological evidence that Neanderthal behaviour was fundamentally different in its conservatism from that of *Homo sapiens sapiens* can be reconciled with the possibility that Neanderthals evolved in an almost leisurely fashion into modern man remains to be seen. □

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Molecular technology

Designing proteins and peptides

from Carl Pabo

ADVANCES in DNA synthesis make it possible to change specific amino acid residues in a protein (by directed mutagenesis) or to create new proteins by synthesizing the required gene. These advances have led to recent discussions about directed modification of protein structure and function[1] and to speculation about designing novel proteins that could be used as 'molecular machinery' for a futuristic microtechnology[2]. Even though we are unable to predict reliably three-dimensional structures from a knowledge of amino acid sequences, there are already examples of the modification of the sequence and structure of peptides in a rational manner. A corresponding ability to modify existing proteins or to design new proteins would have very important theoretical and practical implications.

Several reports show that the rational modification of peptides, based on secondary structure predictions and model-building, is now feasible. Synthetic peptides have been made which retain biological function and appropriate secondary structure, even though they have a very limited sequence homology with the natural peptide[3] or are much smaller[4]. For example, several studies with hormones have indicated that it is possible to stabilize a β-turn by cyclization of the molecule, either by introducing a disulphide bond[5] or by designing a cyclic peptide[4]. The approach has yielded some peptides which are more active and longer-lasting than the natural peptides. For example, the replacement of nine residues of somatostatin with a single proline residue[4] gives a cyclic hexapeptide which is significantly more active than somatostatin itself.

Amphiphilic synthetic helices provide another example of rational peptide design based on considerations of secondary structure. Such helices mimic the biological action of apolipoprotein[6] or the action of melittin[3], even though these helices have very limited sequence homology with the natural protein or peptide. The analogue of melittin differs from the normal sequence at 15 of the first 20 residues (although 6 of the changes are rather conservative) and is homologous only in the C-terminal hexapeptide region (residues 21–26). In spite of these changes, circular dichroism studies suggest that this synthetic peptide forms an α-helix, and the peptide is more active than melittin in lysing erythrocyctes.

At least one ambitious attempt to design a larger peptide, that would contain a mixture of secondary structures, has been reported[7]. A 34-residue polypeptide was designed so that it might form two antiparallel β-strands and an α-helix. The expected arrangement of the backbone and the amino acid sequence were chosen so that the peptide monomer might bind to the anticodon of $tRNA^{Phe}$. The peptide was synthesized, and it appeared to have some affinity for RNA, since a peptide dimer (covalently linked by disulphide bonds) had some RNase activity on a poly(C) substrate. Unfortunately, no detailed structural information was available, so it was not possible to evaluate the accuracy of the structural prediction.

What is the prognosis for modifying and designing proteins? Ulmer has recently discussed the prospects for directed modification of protein structure and function[1]. He sidesteps the protein-folding problem by restricting the study to cases where the three-dimensional structure of the protein has been determined by X-ray crystallography and then considers the prospects for planning sequence changes that would give changes in the activity or physical properties of the protein.

Drexler[2] speculates that it should be possible to design novel proteins and that such proteins could provide a 'general capability for molecular manipulation'. He points out that it may not be necessary to solve the protein-folding problem before we are able to design proteins. The protein designer can choose from an extremely large number of possible amino acid sequences. (A 100 residue protein could have any one of 20^{100} sequences.) Even if only a vanishingly small percentage of these possible sequences give a predictable fold, it might be possible to design proteins, since the engineer could choose to work with this small subset of sequences.

How might one find any rare sequences which would give a predictable fold? Drexler does not discuss this point, but one might proceed, as Gutte *et al.*[7] seem to have done, by designing a fold for the protein backbone before picking an amino acid sequence. Thus, rather than starting with an amino acid sequence and then predicting the conformation of the folded polypeptide, one starts with a conformation of the backbone and then picks an amino acid sequence that should stabilize it. This 'inverted' approach might be useful, because we know more about the characteristics of folded proteins than we do about the process of folding. The approach should allow all of the principles gleaned from X-ray crystallography and structural analysis of proteins to be used directly. For example, knowledge of the secondary structure of the 'prefolded backbone' could be used in the same way that it has been used in the design of peptides. If a section of the backbone has been assigned to an α-helical conformation, one would only add residues with high helical potential. However, the real advantage of the 'inverted' approach, and this is a feature which Gutte *et al.* do not mention, should come when tertiary interactions are considered. 'Inversion' eliminates the problem of predicting long-range interactions, since residues which will interact in the tertiary or quaternary structure are already close in space when they are added to the pre-folded backbone. One knows which residues will be close in three-dimensional space, and one should be able to pick residues which will have favourable interactions with their neighbours.

It may be difficult to design proteins which carry out particular functions, but the use of a pre-folded backbone should also be helpful at this stage. In fact, the inverted approach may simplify protein design even after the folding problem is solved. Since the final folded structure of a protein must always provide the basis for predicting or modifying the function, it would seem most efficient to start with the backbone in a particular configuration and then choose a sequence which gives the desired arrangement of reactive residues. □

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