Abstract

Tolerance and Intolerance in Protein Structure and Function

The combination of directed mutagenesis with high-resolution structure analysis has made it possible to systematically address fundamental questions of protein folding and stability. Some recent results in this area based on studies of the lysozyme of bacteriophage T4 will be briefly reviewed. Studies of a variety of mutant proteins suggests that less than 50% of the overall amino acid sequence protein may be necessary to define the three-dimensional structure of the protein. It is the internal residues that seem to be most important for folding and stability (although not necessarily for function). Cavities within the protein can be designed with different degrees of polarity to provide general tests for docking algorithms. Attempts to analyze and predict the binding of ligands to such cavities have been very encouraging. The protein is not only tolerant of point substitutions but will also accept insertions and deletions. In general, amino acid substitutions result in quite small changes in the structure which are localized to the vicinity of the amino acid substitution. Special classes of mutation can, however, be designed to propagate substantial conformational changes over distances of 20-30 Å. If time permits a brief discussion will be included of the advantages and disadvantages of flash-freezing in macromolecular crystallography. This procedure has become essentially routine but can introduce possible artifacts particularly in the locations of long side-chains in the vicinity of intermolecular contacts. For entropic reasons such side-chains tend to be somewhat disordered at room temperature but can form extensive hydrogen-bonded networks on cooling. Annealing experiments with crystals of E. coli beta-galactosidase suggest that the optimal conditions for flash-freezing are those at which the contraction of the cryo-solvent and of the protein match the reduction in volume of the unit cell.