03. X–5 STRUCTURAL DETERMINATION OF THE COMMON BINDING FEATURES OF SODIUM CHANNEL NEUROTOXINS. By Penelope W. Codding, Departments of Chemistry and of Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta, T2N 1N4, Canada.

Four naturally occurring, lipid-soluble compounds are used in pharmacological tests to probe the mechanism of the passage of sodium ions across membranes in electrically excitable nerve and muscle cells. These four neurotoxins, aconitine, veratridine, grayanotoxin and batrachotoxin, come from different plant or animal sources; yet, all exhibit the same effect on sodium channels. These toxins affect the activation or opening step in the overall ion transport process causing the membrane to remain depolarized. Structure-activity studies on the toxins have shown receptor specificity. Binding studies with radiolabeled batrachotoxin have shown that the toxins bind competitively at a single receptor site. A full characterization of the molecular interactions of these molecules with their common receptor may explain some of the molecular events involved in gating ions through channels.

The results of X-ray crystal structure analysis of aconitine, grayanotoxin III, α-dihydrograyanotoxin II, and veratridine have been compared to batrachotoxin (Karle and Karle, Acta Crystallogr. Sect. B (1969) B25, 428-434) to determine the common structural components that enable them to bind to a single polypeptide site. The polycyclic, inflexible backbones of these toxins produce a fixed conformation for each compound type. This characteristic has been used in molecular superpositions to construct a map of the space occupied by these ligands. The model developed by this method explains much of the structure-activity data for the neurotoxins and suggests some of the amino acid residues present in the activation receptor site.
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03. X–6 **A Structural and Biological Analysis of Cardiac Steroids and Their Glycosides,** D. C. Rohrer* D.S. Fullerton, K. Ahmed and A.H.L. From, Medical Foundation of Buffalo, Inc., Buffalo, NY 14203 USA; Oregon State University; Corvallis, Oregon 97331 USA, Veterans Administration Medical Center; Minneapolis, Minnesota 55417 USA

Cardiac steroids represent a major class of medications widely used in the treatment of congestive heart failure. They are also specific inhibitors of Na^+,K^+-ATPase, the enzyme which mediates the cellular sodium pump and has been considered as the putative receptor for these drugs.

The structural and conformational characteristics of a series of cardiac steroid analogs (genins) and their glycoside derivatives have been determined from analysis of crystal structure results and molecular mechanics calculations in order to explore the relationship between their structure and biological activity. The A, B and C rings in the steroid backbone remain essentially conformationally invariant in all the structures, while the D rings show a high degree of flexibility. The conformational characteristics of the C17β side groups on the various analogs do not seem to be significantly affected by the nature of the C3 glycoside substituent. The bonds linking the steroid to the sugar moieties show a surprisingly small range of rotational freedom. The C2'–C1'–O3–C3 torsion angles range only over 29.4° in ten crystal structures, while the C1'–O3–C3–C2 torsion angles range over 115.6°. A comparison of the structural characteristics of the "active" conformations of these analogs and their derivatives with their potency as hog kidney Na^+,K^+-ATPase inhibitors, reveals that the same type of linear relationship observed for the genins exists for the glycoside derivatives. Thus, when the positions of the carbonyl oxygen on the C17β side group of the cardiac steroid analogs relative to that oxygen on digitoxigenin are compared to their strengths as Na^+,K^+-ATPase inhibitors using regression techniques, a linear correlation with an r^2 of 0.93 is observed. The addition of the sugar substituent, however, enhances the potency over that of the genins in a structurally specific way. The potencies of those analogs derivatized with a sugar, such as β-D-galactose, containing a 4'-axial hydroxyl group or a blocked 4'-equatorial oxygen sugar, such as β-D-digitoxose acetonide, have their inhibition strengths increased systematically by a factor of about 2, while those derivatized with a sugar, such as β-D-digitoxose or β-D-glucose, containing a 4'-equatorial hydroxyl have their activities increased by about a factor of 10. These results indicate that the orientation and nature of the O4' substituent on the sugar moiety of the cardiac glycosides is directly involved in the enhancing process.

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03. X–7 ENZYME ENGINEERING FROM CRYSTALLOGRAPHICALLY KNOWN STRUCTURES. By D.M. Blow, Biophysics Group, Blackett Laboratory, Imperial College, London SW7

The new techniques of protein engineering make possible a wide range of experiments of enzymes, which are meaningful only if a precise three-dimensional structure is known. Tyrosyl-tRNA synthetase has been used for model experiments of several types:

1. Direct measurement of interaction energy of a substrate with a particular amino-acid side chain.
2. Demonstration of a specific catalytic role for a particular amino-acid side chain.
3. Alteration of enzyme specificity.
4. Study of the effects of a small structural change (involving more than an amino-acid side chain) on enzyme properties.
5. Demonstration of different functional roles for different domains of an enzyme.
6. Study of effects on enzyme properties of changes in quaternary structure.
7. Effects of these changes on protein folding.

In another system, experiments are in progress for:

8. Modification of enzyme stability - e.g. for use in the industrial environment.

In the long term, one can envisage:

9. Design of new functional enzymes, perhaps by construction from fragments of proteins of known structure.

A thorough interpretation of the results of these experiments will require crystallographic determination of the structures of the engineered proteins. Our understanding of protein structure and enzyme mechanism can be measured by our ability to predict the outcome of enzyme engineering experiments, both the structure of the engineered enzyme, and its physical and enzymatic properties.