Applications of the PROPHET system in correlating crystallographic structural data with biological information*

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The goal of the Molecular Biophysics Department at the Medical Foundation of Buffalo is to establish relationships between the physical structures of molecules and their biological activities. At present, the steroid and thyroid hormones, their derivatives, analogs, and inhibitors are the materials of major interest in this research, but work on other groups of biologically active molecules is anticipated or in progress. The PROPHET system** provides a powerful medium for the assembly, storage, and analysis of both structural and biological data. Correlative studies of these two types of data are particularly important because they may lead to an understanding of the molecular level mechanisms of action of hormones, drugs, antibiotics, and other biological substances. The PROPHET system is well suited for this type of analysis because it permits communication and interaction among scientists who are experts on various phases of molecular biology.

The investigation of structural-functional relationships as it is being pursued at the Medical Foundation may be divided into the following stages: (1) The basic structural information must be collected and stored within the PROPHET system. X-ray crystal structure determinations in this and other laboratories have provided a large volume of molecular structural information in the form of atomic coordinates. Any geometric parameter of interest such as interatomic distances, bond angles, torsional angles, and deviations from least-squares planes can be calculated from these coordinates. The coordinates for 200 steroids, 50 aromatic amino acids, and 25 thyroactive compounds have been stored within the PROPHET system. (2) The geometrical data for groups of similar substances are examined for correlations between structural features and chemical or biological properties. (3) An attempt is made to explain the mechanism of action of the molecules of interest in terms of any observed correlations. (4) Speculation is made concerning what changes in the molecular structure may be possible and what effects these changes will have on the biological action of the molecule. The present paper will illustrate some of the ways in which the PROPHET system has been used to compare conformational features of related molecules and to correlate the structural differences with differences in chemical and biological properties.

The atomic coordinates and related information for each molecule in the data bank are stored in disk files where they may be accessed either by FORTRAN programs or PROPHET procedures. A PROPHET variable of type MOLECULE is also created for each structure. A pictorial representation of a molecule may be created either by sketching a chemical diagram on the tablet or by executing a PROPHET procedure which uses the crystallographic coordinate file to determine the positions of the atoms and chemical bonds. Both methods generate a connection table, and some miscellaneous information such as the molecular weight and percent composition is also computed. PROPHET can make the equivalent of a three-dimensional Dreiding model for acyclic molecules and for simple cyclic compounds containing rings which are included in its ring dictionary. Figure 1 shows sketches of the steroid hormone cortisol and the thyroid hormone triiodothyronine (T3). Figure 2 illustrates the three-dimensional model of T3 computed from the sketch as well as a view of the actual crystallographically observed structure. At present, a three-dimensional model cannot be computed for the fused ring system comprising the steroid nucleus. For obvious reasons, the sketching feature is used only when experimental coordinates are not available or for drawing a molecular fragment which is to be used in a substructure search.

The TABLE and GRAPH functions of the PROPHET system may be used to analyze information derived from the coordinates and to arrange this information so that statistics may be computed in a convenient fashion. One conformational feature of the thyroactive compounds which was analyzed in this way is the disposition of the planar aromatic rings about the connecting oxygen atom. The gross conformational changes in the molecule which accompany rotations about

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** A detailed description of PROPHET System features and organization is given in the accompanying paper by Castleman, et al.
the bonds involving the central oxygen atom are depicted in Figure 3. All reasonable parameters which could describe the relative orientation of the two rings in the 17 thyroactive structures were stored in a table (Table I). Plots were made of various combinations of rows and columns in order to look for systematic patterns. Figure 4 is an example of a graph where no correlation between the parameters was observed. On the other hand, Figure 5 shows a discontinuity with the data falling into two widely separated groups, and Figure 6 illustrates the two overall types of molecular conformations which have been termed transoid and cisoid.

One of the most convenient features of the PROPHET
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**TOTAL DISTRIBUTION OF TORSIONAL ANGLES IN THYRONINE STRUCTURES**

![Graph showing distribution of torsional angles in thyonine structures.](image)

**Figure 5**

**Figure 6**

**Figure 7**

**Figure 8**

**Figure 9**

system is the case with which a molecule may be viewed from different perspectives. Figure 7 shows the cortisol molecule in orientations which may be described as top, side, and end views. These pictures were produced using the TURN commands which rotate displays about the three axes.

The experimentally observed conformation of a molecule may also be modified using simple commands so that geometrical parameters of a theoretical conformation can be quickly computed. Examination of these parameters may reveal energetically unfavorable features of the theoretical conformation. For example, the orientation of the side-chain attached to C(17) in the cortisol nucleus might be thought to be flexible, but crystallographic investigation of cortisol derivatives has shown the torsional angles about the C(17)-C(20) bond to be largely invariant. As shown in Figure 8, the CHEMSET command was used to rotate the side-chain about this bond, and distances were then calculated between atoms in the nucleus and the side-chain by simply pointing to the atoms in question with the stylus. This feature of the PROPHET system makes it easy to deform a molecule by small amounts and to follow the atomic interactions at many different points.

A comparison of the structural data for cortisol and its derivatives also reveals one of the best examples of a correlation between conformational differences and biological activity which has been observed in the steroids. The effects of cortisol on carbohydrate metabolism and inflammatory re-
actions may be enhanced by a number of modifications to the steroid nucleus including dehydrogenation of atoms C(1) and C(2) and by replacement of the hydrogen at the 9-position on the bottom or α-face of the molecule with a fluorine atom (see Figure 9). A comparison of the molecular geometry of the 9α-fluorocortisol molecule with the structures of cortisol and 6α-methyl-1-dehydrocortisol suggests that the increased activity of the 9α-fluoro derivative may result, in part, from unexpected changes in the A-ring conformation. Superposition of these molecules in Figure 10 shows that the A-ring in 9α-fluorocortisol is bent underneath the plane of the molecule to a much greater extent than is the A-ring in cortisol, and in this respect, the conformation of 9α-fluoro-
cortisol resembles the conformation of 6α-methyl-1-dehydrocortisol. From inspection of a Dreiding model of 6α-methyl-1-dehydrocortisol, it can be seen that the presence of the 1-2 double bond will force the A-ring to adopt a conformation similar to that actually observed in the crystal structure. However, the reason for the sharp bowing of the A-ring toward the α-side in 9α-fluorocortisol is not so obvious, and the bowing was not anticipated prior to the crystallographic investigation. These differences in A-ring conformation influence the distances between O(3) and other oxygen atoms in cortisol and its derivatives as shown in Figure 11. Variations of this type may be related to the ability of these molecules to bind to proteins in vivo.

The preceding illustrations demonstrate the utility of the PROPHET system in the search for correlations between molecular structure and biological function. The investigation of such structural-functional relationships will be greatly simplified when files of bioassay data become available within PROPHET. Greater use of PROPHET to explore mechanisms of action and to identify the effects of changes in structure will also be made in the future. Molecular model building experiments and conformational energy calculations are some of the techniques which will be used to accomplish these goals. For example, rotameric potential energy mapping procedures can be employed to make a sophisticated analysis of the interactions which occur when a molecule is deformed as was illustrated by rotation of the cortisol sidechain. These procedures produce energy contour maps which relate various molecular conformations to the energy associated with nonbonded contacts between the atoms at the ends of rotating bonds. The interactions of more than one molecule will also be investigated in order to provide models for such molecular events as the binding of a steroid hormone to its receptor protein.