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COMPUTER-AIDED DRUG DESIGN

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INTRODUCTION

Molecular modeling and computational chemistry are assuming an increasingly important role in understanding the basis of drug–receptor interactions and assisting the medicinal chemist in the design of new therapeutic agents. Computer graphics has emerged as a cost-effective tool, and adequate computational power is now available, which removes limitations that have crippled computational chemistry. These advances have stimulated the development of software tools for probing the three-dimensional aspects of specificity. Several recent reviews by Cohen (1, 2), Hopfinger (3), and Marshall & Motoc (4) offer more detailed coverage of this area.

To gain insight into the application of these approaches, we focus on recent studies and emphasize the range of techniques. Which technique is chosen is dictated by the knowledge of the molecular therapeutic target, whether enzyme or receptor. We emphasize the inherent limitations of each technique so that the reader may temper his enthusiasm in the face of the masterful and, sometimes artistic, applications of these approaches.

KNOWN ACTIVE SITES

Detailed information on the three-dimensional structure of macromolecules is available for an increasing number of enzymes and nucleic acids (the latest edition of the Brookhaven database contains over 290 structures). With the rapid developments in genetic engineering, it is possible to isolate and clone enough target macromolecules for experimental investigation. The increased speed of new detectors in X-ray crystallography [along with advances in nuclear magnetic resonance (NMR) spectroscopy that allow the determination of three-dimensional structure on noncrystalline materials] places an increas-

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ing emphasis on the determination of the three-dimensional structure of the target as the prelude to rational drug design. The three-dimensional solution structure of a 75-residue protein, tendamistat, determined by NMR, resembles closely its crystal structure, which was determined independently (6). The determination of this structure in solution marks the beginning of a new era.

The Wellcome group [see reviews by Beddell (7) and Goodford (8)] pioneered the designing of compounds to effect the oxygen dissociation of hemoglobin based on the known allosteric effector, 2,3-diphosphoglycerate (DPG). The three-dimensional structure of the complex has inspired a number of other such efforts. The work of the Abraham group on sickle cell anemia (9) resulted in the design of novel chemical structures with good affinity that were subsequently shown to bind as predicted by determination of the crystal structure of the drug–hemoglobin complex. In both of these studies, however, the predictions were qualitative and based on placing groups on an appropriate three-dimensional framework for potential interaction with complementary groups in the receptor. Analogs with enhanced affinity for dihydrofolate reductase (DHFR) have also been designed by Kuyper et al (10) using a similar rationale.

In cases such as these, computer modeling has simply replaced the physical model of the enzyme that formed the basis of the original work. Binding affinity is estimated by counting the enhanced interactions (e.g. hydrogen bonds) and multiplying this number by some average energy of interaction, which should approximate the enhanced affinity. Compounds have also been designed to affect the solubility of insulin by influencing the crystal structure (11). Andrews et al (12) analyzed the binding affinities of a large set of drugs to partition these affinities into groups based on average interactions for each functional group. These average values provide a scheme to evaluate how well a drug binds to its receptor. If the observed affinity is greater than that predicted from a summation of average fragment contributions, then the drug–receptor complex must have greater than average complementarity. Such analyses may also provide some insight into entropy loss on binding (13). Goodford (14) developed a method of identifying optimal sites for interaction by probing the surface of known structures with different chemical fragments. This method correctly identifies known sites for bound water and other ligands. Kuntz et al (15) developed an efficient procedure to determine complementarity between rigid ligands and potential binding clefts on known crystal structures. This procedure generates a set of spheres that fill the pockets and the grooves on the surface of a receptor molecule. These spheres are then subdivided into sites. A similar representation is used for the ligand. Correspondence that does not require explicit rotation and translation of one

structure into the other is sought between the distance matrixes for the sets of spheres representing the ligand and the presumptive site.

Hansch and coworkers have compared the parameters derived from quantitative structure-activity relationship (QSAR) analyses of ligands that bind to enzymes. The structures of dihydrofolate reductase (16), papain (17), actinidin (18), chymotrypsin (19), trypsin (20), carboxypeptidase (unpublished work cited in 17), alcohol dehydrogenase (22), and carbonic anhydrase (23) have been determined. Generally the terms derived in the QSAR equations do bear a relationship to the three-dimensional structure. In spite of the flexibility in conformation available to enzymes, the data obtained in solution and summarized by the correlation equation agree quite well with the static view of the active site. In QSAR, steric effects are often modeled by continuous variables that show that contact between the ligand and the enzyme does not preclude activity, but rather reduces it in proportion to the size of the substituent. The coefficient of the hydrophobic term, log P or pi, in the QSAR equation can be roughly related to the extent of desolvation of the ligand. A coefficient of 1 would suggest binding in a pocket, while a coefficient of 0.5 might suggest binding of the substituent to a flat surface requiring only partial desolvation. QSAR can offer insight, therefore, into the nature of the receptor site adjacent to the substituent under study.

A word of caution comes from the studies by Perutz et al (24) of the crystal structure of hemoglobin complexed with four compounds, each of which affects thc polymerization of deoxyhemoglobin S. These compounds bound at different sites between the alpha chains far from the DPG site, implying that the same molecular event, a change in the allosteric equilibrium of the receptor, can occur by different molecular mechanisms. The binding sites were generally characterized as niches in the protein with available van der Waals space and complementary electrostatic interactions. The binding site of *p*-bromobenzyloxyacetic acid, however, was located in a position closely packed with sidechains in the uncomplexed structure. This observation emphasizes the dynamic nature of protein structure and the ability of drug-receptor interactions to induce complementarity.

Qualitative Applications

Vedani & Meyer (25) qualitatively analyzed the interactions of 28 sulfonamide inhibitors of human carbonic anhydrase, and suggested two binding modes for the heterocyclic ring in the active site. A hydrophobic pocket more than 10 Å from the active site zinc contributes important binding interactions. Smith et al (26) examined the mechanism of action and inhibition of thermolysin as a prelude to inhibitor design and more quantitative studies. DNA has been intensively targeted as a binding site. Many antibiotics such as actin-

omycin D and daunomycin bind by intercalation; the helix expands so that the flat polycyclic drug can occupy a site between two adjacent base pairs. Hendry and coworkers (27) postulated a similar mechanism to explain the hormonal activity of steroids. Other antitumor drugs bind within the minor groove of the DNA double helix (28). Kopka et al (29) determined the crystal structure of a netropsin-B DNA double-helix complex. Based on the mode of binding observed, Goodsell & Dickerson (30) analyzed the geometrical requirements for complementarity with the minor groove for 14 candidate monomer units to determine the optimal length for the oligomeric drug.

Thermodynamics of Binding

A second level of sophistication develops when one attempts to calculate the affinity of the ligand for the presumed site. Molecular mechanics is used to calculate the energy of the complex; from this number the energy of the free ligand and the free receptor are subtracted. The difference is the approximate enthalpy of binding. To understand the problems associated with these calculations, a short digression is in order. The first problem arises from the fact that calculations often approximate the enthalpy (ΔH) of binding in vacuo, when the quantity that should correlate with experimental observation is the free energy (ΔG) of binding in solution. The omission of solvation and entropy effects in theoretical approach of most studies is clearly an error, and reflects inadequate methodology for their estimation. A recent paper by Lybrand et al (31) using molecular dynamics with the explicit inclusion of the solvent and calculation of the entropic effect shows agreement within experimental error for the selectivity of the chloride complex of a macrotricyclic compound SC24 compared with the bromide complex. Considering the computational complexity of this approach, one cannot yet judge its practicality for large molecules in the near future. A preliminary report on trypsin by Wong & McCammon (32) is encouraging, but longer simulation is necessary to evaluate the agreement with experimental data. The assumption that entropy and solvation effects are similar for analogs is often used to justify comparison of the differences in binding activity between analogs, or the $\Delta\Delta$G. Another effect that makes correlations between calculated ΔH and binding affinities likely is the entropy–enthalpy compensation seen in aqueous solvents (33). Because of this relationship between the two components of free energy, one of the components should correlate with the other, with appropriate scaling.

These problems exist regardless of the quality of the force field used in the energy evaluation. Unfortunately, several reservations about current application of molecular mechanics must be mentioned. Parameters for novel structures are not readily available and require extensive theoretical or experimental study. In addition, the effects of electrostatics at the molecular

level are unclear unless one can apply quantum mechanics in a fairly rigorous manner. The dielectric constant that screens the strength of interaction is, in fact, a variable that depends on the intervening atoms and their polarizability (34, 35). Currently the popular approach is to use a distance-dependent dielectric, 1/R, which simplifies the computation by eliminating a square root calculation. These practical limitations cause uncertainty in the interpretation of calculated binding affinities. Reliable accuracies of the order of a Kcal/mole would still lead to inaccuracies in predicted potency of a factor of ten, since at room temperature, 1.4 Kcal/mole reflects a tenfold concentration difference in binding affinity.

Applications

The binding of the thyroid hormones, T3 and T4, to prealbumin has been studied extensively as a model system, as the crystal structures of the complexes have been determined. Oatley et al (36) used the AMBER force field to refine the energy of the complexes. The calculated relative energy of binding (T4 to T3) was −2.9 Kcal/mole after a simple empirical correction for the solvation differences of the two hormones, whereas the observed differences are −1.4 Kcal/mole. Considering the reservations expressed above regarding the omission of entropy and force field uncertainties, this result is encouraging. Application to a large series of analogs would offer a firmer basis for evaluation. Zakrzewska et al (37) analyzed the binding energy of six antibiotics that bind in the minor groove of B-DNA. Introduction of a correction for solvent effect was essential to reproduce correctly the relative affinity and to scale the calculated energy to an appropriate level. Crystal structure analysis of the netropsin-DNA complex confirms (29) the prediction of the location of the charged nitrogen groups. Caldwell & Kollman (38) modeled this complex using NMR data as a guide. The major discrepancy with the crystal structure was the position of the charged headgroups of netropsin that were closely associated with the phosphate backbone in the calculated structure. This discrepancy reflects the omission of solvent and counterions in this study. Rao et al (39) explored several alternate DNA-binding modes for mitomycins and favor major groove binding for this drug. Lybrand & Kollman (40) compared a united atom force field with consideration of each atom of the intercalation drug, ethidium, with DNA fragments. Excellent agreement with experimental data was found with the all-atom representation. Besides a minimum energy complex corresponding to a model derived from crystal structures, a second minimum with a strong hydrogen bond between a phosphate oxygen and an amino hydrogen of ethidium was observed. This finding may reflect the absence of solvent in the calculations, which compounds the problem with electrostatics.

One major difficulty is the assumption of a common binding mode for

compounds with similar structures. Even if one assumes rigid geometry for both the drug and the receptor, one must still explore six parameters (three translation and three orientation variables) to ensure that the global minimum for the complex has been determined. Adding internal flexibility to both the drug and the site to accommodate induced fit increases the computational complexity to the point that technological limitations preclude systematic search for optimal binding modes. Naruto et al (41) combined systematic search for productive binding modes with energy minimization for a series of mechanism-based inhibitors of chymotrypsin. The calculated enthalpies of binding predicted the order of affinity as well as a lack of stereoselectivity which was subsequently confirmed by resolution and enzymatic assay. The alternate binding modes found for the stereoisomers with equivalent affinity would not have been discovered by minimization from equivalent starting geometries. The success of this study probably reflects both the similar contributions to electrostatics, solvation, and entropy in a congeneric series as well as the enthalpy–entropy correlation referred to above.

RECEPTOR SITE BY HOMOLOGY

In a number of cases of therapeutic interest, the amino acid sequence of the target protein is known and homologous proteins exist whose three-dimensional structure has been determined. Computer modeling has been used to transform the known structure into the target by a combination of sidechain replacement and energy minimization. Obviously, when the structure of the target protein is close to the known structure, one has a greater chance of success. Aspartyl proteases are probably most widely studied. With these structures, renin inhibitors are the target (42). Several investigators have attempted to evaluate the likelihood of success of such studies. The Alberta group (43) made the most critical evaluation. They determined the crystal structure of an enzyme that had previously been modeled by homology. Drug design usually focuses on the active site; specificity for the particular enzyme is a design goal. Unfortunately, it is the residues that differ between the two proteins that cause the specificity. The successful design of renin inhibitors that resulted from this approach may be due to the same medicinal chemical logic that inspired so many angiotensin-converting enzyme (ACE) inhibitors in the absence of three-dimensional information. In that case, mechanistic arguments generated a framework on which to base designs, as the sequence of ACE was not available—even though the structures of carboxypeptidase A and thermolysin might have served as a rough template (44).

RECEPTOR SITE BY INDUCTION

With the advent of DNA sequencing, determining the sequence of proteins by inference has become routine, and a project to sequence the entire human

genome is under consideration. Unfortunately, knowing the sequence of the therapeutic target does not aid the medicinal chemist. Progress in understanding the process of protein folding continues at an enhanced rate, with genetic engineering techniques offering a powerful experimental adjunct to theoretical studies. What hope do we have of predicting the three-dimensional structure based on sequence information alone? Whereas it is clear the tertiary information resides in the sequence, the translation rules have defied definition. Predictive methodology based strictly on statistical approaches is only approximately 60% accurate in secondary structure prediction (45). Even if one could correctly predict secondary structure, the correct folding is a combinatorial problem whose complexity should not be underestimated. A heuristic approach by Cohen et al (46) claims a high success rate (approximately 90%) in turn prediction. Sheridan et al (47, 48) have correlated amino acid composition and hydrophobicity patterns with the structure of protein domains. Their results may allow the prediction of the structural class with some degree of certainty and offer increased hope that such methodology may allow systematic exploration of possible folded structures by energy minimization. The same caveats expressed with regard to force fields, entropy, solvation, and local minima apply, of course, and become even more dominant due to the size of the structures considered.

Finer-Moore & Stroud (49) and Guy (50) proposed models for the acetylcholine receptor alpha subunit that contains the acetylcholine binding site. Mishina et al (51) described 26 analogs of this over-400-residue subunit prepared by site-directed mutagenesis that support the overall features of the models. Several models (52) of the sodium channel have also been proposed based on secondary structure predictions. McCormick et al (53) proposed a model for p21, the product of the *ras* oncogene, which Pincus & Scheraga (54) also studied. Blanar et al (55) used the techniques developed by Cohen et al (46) to model the RecA protein of *Escherichia coli*. A model of the apolipoprotein B-E receptor was developed by De Loof et al (56), who used hydrophobicity profiles to determine protein domains. In each of these cases, many more experimental data are required to refine, and, perhaps, redefine these models.

RECEPTOR SITE BY DEDUCTION

The problem most familiar to the medicinal chemist is the one in which the therapeutic target (the receptor) can by inferred only by binding studies or pharmacological studies. Systematic variation of the chemical structure leads quickly to the conclusion that some parts of the molecule are critical for activity, whereas others can be changed, causing only minor variations in affinity. These qualitative differences in results led to the concept of the pharmacophore at the turn of the century.

The inherent conformational freedom associated with most drugs hampered efforts to interpret structure-activity information in a three-dimensional framework. The work of Hansch and others in developing the QSAR paradigm showed that a common frame of reference based on a congeneric series offered a basis for rational interpretation. Comparison of binding modes at known active sites with the correlation equations developed with QSAR show clearly that the coefficients of the parameters can be interpreted with some degree of assurance in terms of the binding site (22, 23). While this approach is essentially topological, a topographical, or three-dimensional, equivalent must exist. This realization led Marshall and his coworkers (57–59) to develop the Active Analog Approach in which the pharmacophore provides the frame of reference analogous to the congeneric framework as a basis for comparison of molecules.

The pharmacophoric pattern must be defined before congeneric series can be compared. With rigid molecules and sufficient modification, inference of the pharmacophore should be straightforward. Unfortunately, most systems of therapeutic interest do not limit themselves to rigid structures, and the conformational problem must be confronted. Many approaches to this problem have been suggested. Most center around convenience and available methodology. Many have focused on energy minima as the key to biological activity. Certainly, most methodologies such as molecular mechanics, crystallography, or spectroscopy are aimed at determining the minimum under the experimental conditions of the investigation. The absence of the receptor in these studies clearly compromises their relevance on theoretical grounds. Several systems have been studied in sufficient detail to confirm this inadequacy (4). Nevertheless, papers continue to be published in which biological relevance is claimed, when no correlation of any observed phenomena with biological activity has been demonstrated. Hopefully, the availability of receptors by isolation and cloning will allow both experimental determination of the receptor-bound conformation, as well as measurement of difference between the solution ensemble of conformers and those limited sets capable of binding with high affinity.

The determination of the pharmacophore in the Active Analog Approach requires the initial examination of each three-dimensional pattern of candidate functional groups resulting from an energetically accessible conformation. If the premise of a pharmacophore, or common electronic message, is tenable, then each active analog must be capable of presenting that pattern that must appear in the set of possible patterns determined for each compound. Several approaches to pharmacophore identification have been suggested. Naruto et al (60) have applied the concept of constrained minimization of active compounds (in which the pharmacophoric groups are forced to assume a similar geometric arrangement) to histamine antagonists. Sheridan et al (61) cleverly

apply distance geometry and use the simultaneous constraints presented by an ensemble of active molecules to find common geometric patterns, as demonstrated with the nicotinic pharmacophore. Both of these paradigms have the inherent limitation of minimization procedures that find a solution without regard to uniqueness, and that depends on the formulation of the problem. Multiple applications with different starting points can indicate the validity of the solution. Systematic exploration of the conformations available to an active compound determines the set of possible three-dimensional patterns that the pharmacophoric groups can present. Finding this set of patterns requires determining the logical intersection of the patterns available to each of the active compounds.

One must make an arbitrary decision regarding the energetics of conformations rejected from consideration. In some cases, the conformation presenting the pharmacophore may be near a local minimum energetically, but no a priori assumptions exclude perturbation of the energy surface of the isolated drug by the binding interaction. For example, 28 different chemical classes of ACE inhibitors were determined (D. Mayer, I. Motoc, C. B. Naylor & G. R. Marshall, unpublished information) to be capable of binding to a unique active site with a maximum energy distortion of 4 Kcal/mole upon binding. In other words, if the energy cutoff for consideration had been set at less than 3 Kcal/mole, then no active site would be capable of optimally binding these inhibitors. Conformers less stable than 2 Kcal/mole have little chance of being detected by most experimental techniques, as their abundance is less than 1% at room temperature.

Once a pharmacophoric hypothesis is proven valid by the experimental data, it can be further tested for consistency by examining compounds that have the prerequisite functional groups, but show little or no activity. Each compound can be checked to see if it can assume an energetically reasonable conformation in which the pharmacophoric groups are correctly aligned for activity. For those in which such alignment is possible, alternative explanations for inactivity must be sought. These explanations could include differences in distribution or metabolism, as well as negative steric interaction with the receptor. The technique of receptor mapping has been developed (59) to determine the volume adjacent to the pharmacophore that must exist for drug binding. Addition of the volume essential for each active drug when bound appropriately determines the minimal available space. One plausible explanation for inactivity arises when an inactive compound, capable of presenting the pharmacophore, requires a novel volume. Part of this novel volume may be occupied by the receptor and may preclude binding of the compound in question.

This approach suffers from several limitations. First, analysis of crystal structures of enzyme–ligand complexes shows clearly that alternate binding

modes often exist, and that the hypothesized overlap of functional groups of the ligand is an oversimplification. If the data are sufficiently diverse, assumptions can be made with regard to the types of functionality in the receptor responsible for binding. Then, supermolecules can be constructed with such groups attached to those complementary groups of the ligand by idealized geometry. The investigator can search systematically for all geometrical arrangements of the hypothetical receptor site capable of interacting with the set of ligands. In the case of ACE, whose three-dimensional structure has yet to be determined, D. Mayer, I. Motoc, C. B. Naylor & G. R. Marshall (unpublished information) determined a unique active-site geometry for the postulated zinc, hydrogen bond donor, and positively charged groups based on 28 different chemical classes of inhibitors. For each chemical class, the energy difference between the bound conformer and the nearest local minimum was determined by energy minimization, with 4 Kcal/mole being the maximum decrease. In the pharmacophoric model of Wender et al (63) for the activation of protein kinase C, the conformation of 1,2-diacylglycerol corresponding to the C-4, C-9, and C-20 hydroxyl of phorbol is approximately 4 Kcal/mole above the global minimum. Previous efforts to determine the relative geometry of the active site of ACE were limited to a few chemical classes, and focused extensively on energetic minima to limit the computational complexity of the problem (64–66).

Knowledge of the chemistry at an enzyme active site often places severe stereochemical constraints on the relative orientation of groups. These restraints simplified greatly the consideration of possible binding modes in the analysis of mechanism-based inhibitors of chymotrypsin (41). Lim & Spirin (67) analyzed the stereochemistry of transpeptidation on the ribosome. They determined that a unique conformation of the tetrahedral intermediate is consistent with the formation of each of the 400 pairs of amino acids possible, which implies a particular geometry for the active site.

Another limitation concerns the lack of quantitation and the loss of information sustained by ignoring the relative affinities
pounds. A method for three-dimensional quantitative structure-activity studies remains to be completed. Several approaches being developed merit attention. A logical extension of the receptor-mapping approach by Hopfinger (68) begins with a congeneric series and relates overlap in molecular volume for the assumed receptor-bound conformation to other QSAR parameters to establish a correlation equation. Extensions (69–70) of this approach have focused on overlaps in the potential fields generated by probing the volume adjacent to the receptor with various probes, such as proton, water, and methyl. The loss of geometrical information by integration of the field is an inherent limitation in this procedure that defies the experience in specific directional interactions obtained by analysis of receptor-drug complexes. Wise et al (71) developed an approach that overcomes this objection. In this

approach the potential field is sampled on a lattice adjacent to the drug. The lattice is oriented according to a set of rules, i.e. a pharmacophore hypothesis. A correlation is then sought by statistical evaluation of combinations of grid point values. While the number of parameters involved probably assures that such a correlation will be found, newer statistical methods developed by Wold et al (72) allow one to analyze this problem with some security. The only published application involves inhibitors of GABA uptake (73).

Motoc & Marshall (74) have also extended the volume mapping approach in a quantitative manner by subdividing the volume occupied by a drug and classifying each segment according to its contribution to affinity. This approach combines a recognition of the positive contribution to affinity by occupancy of the receptor site, as well as of negative contributions by competition with the receptor for volume and of a neutral portion that does not change its solvation state on binding. Only fragmentary aspects of this approach have been published (75), and an application of the completed procedure is not yet available.

In a quite distinctive approach, Ghose & Crippen (76) used the methodology of distance geometry and the concepts of QSAR to generate a hypothetical site with properties whose calculated affinity for sets of analogs reproduced the experimental affinities. This approach, however, does not appear to be deterministic and requires interaction and guidance from the user to devise a model active site. Nevertheless, such a site model offers help in the selection of compounds for screening and synthesis as well as an opportunity for continued refinement as new data are obtained. It appears a more refined approach than that constructing a hypothetical receptor site analogous to that advocated by Kier (77) or those built from amino acid fragments by Holtje & Tintelnot (78) and whose affinity for drugs correlated strongly with experimental data. Linschoten et al (79) developed a nine-point geometric representation of the turkey erythrocyte beta receptor with six energy parameters whose affinity for 58 diverse structures correlated well with observed affinity. With this representation these authors predicted different binding sites for the phenyl rings of phenethanolamines and phenoxypropanolamines.

The concept of pharmacophore, or three-dimensional mimicry, has guided the interpretation of activity of diverse chemical compounds as well as the development of several new classes for therapeutic development (1-4). Lloyd & Andrews (80) published a provocative study in which a common pharmacophoric mode was found for 14 classes of CNS-active drugs. A common precursor biogenic amine receptor could be responsible for this common binding mode, and different receptors could distinguish between molecules based on differences in accessory binding sites. Jeffrey & Liskamp (81) explained the tumor-promoting activity of phorbol esters, teleocidin B, and aplysiatoxin by the pharmacophoric concept. Wender et al (63) have

analyzed similar compounds, including the natural activator of protein kinase C, 1,2-diacylglycerol. Based on the pharmacophoric model deduced, two novel structures with the predicted activity were prepared. Griffith et al (82) developed a novel atypical antidepressant based on a three-dimensional model of classical tricyclic antidepressants and mianserin. Martin & Kim (83) described the application of molecular modeling and QSAR reasoning that aided the development of a new class of diuretics (84). Loew and coworkers attempted to define receptor-site requirements that would explain activity at the benzodiazepine receptor (85, 86). The recent development of a nonpeptide antagonist of cholecystokinin by Evans et al (87) led to the suggestion that the benzodiazepine ring may exploit some feature common to peptide receptors. Two attempts (88, 89) to rationalize dopamine structure-activity relations based on receptor-site models have appeared.

On the other hand, emphasis on minimum energy structures continues in the literature. Robson & Finn (90) describe an approach that includes solvation effects, but still dwells on minimum energy conformers as exemplified in work on thyrotropin-releasing hormone (TRH) (91). Momany & Chuman (92) reviewed their work on morphiceptin and enkephalin analogs. These small peptides received attention from Loew et al (93), Hall & Pavitt (94), Maigret et al (95), and Paine & Scheraga (96). Using the Active Analog Approach, Nelson & Marshall (97) and Nelson et al (98) have clearly defined the receptor-bound conformation of morphiceptin by the use of analogs containing amino acids with defined conformational constraints. Font (99) also defined three alternatives for the receptor-bound conformation of TRH. Wong et al (100) presented their conformational analysis on a set of anticonvulsant drugs without consideration of a previous similar study by Klunk et al (101) of a different set of anticonvulsant compounds.

DETERMINATION OF SOLUTION CONFORMATION

The recent advances in NMR spectroscopy added to algorithmic advances in combining constraints from experiments with conformational calculations will clearly have a dramatic impact on drug design. Experimental measurements of spin-spin coupling constants, nuclear Overhauser effects (NOE), and hydrogen bonding determine sets of distances within a molecule that must be satisfied in any model. In the case of the small protein, tendamistat, 401 distant constraints from NOE measurements, 50 torsion angle constraints, and 168 distance constraints from hydrogen bonds and disulfide bridges were used (5). Rigid geometry was assumed to determine the three-dimensional structure according to the method of Braun & Go (102). Havel & Wuthrich (103) developed a similar methodology around the distance geometry paradigm.

Clore et al (104) explored the use of molecular dynamics with experimental constraints in determining the solution conformation of DNA binding F helix of cAMP receptor protein of *Escherichia coli*. In this case, 87 approximate distances from NOE measurements were used to define the alpha helical structure of this 17-residue fragment.

The three-dimensional structure of a novel antibiotic, aridicin A of the vancomycin-ristocetin family, was determined by a combination of two-dimensional NMR, systematic search, and energy minimization (105). In this case, the stereochemistry was also assigned in an unambiguous manner. The structure was determined as the only one consistent with the experimental data by systematic examination of conformational possibilities. Mildvan and coworkers (106, 107) pioneered the substitution of paramagnetic ions in metallo-enzymes as a probe to determine the enzyme-bound conformation of substrates and cofactors as well as the active-site residues adjacent to the metal. Two recent examples are the ATP-binding site of adenylate kinase (106) and the conformation of glutathione analogs bound to glyoxalase I (107).

METHODOLOGY

The application of various approaches is intimately related to the current stage of methodology development. Any comments are only relevant to the current state, as reflected by the experience of the author. Newer developments which may impact the applicability will be stressed.

Molecular Mechanics

The lack of parameters for many molecular fragments limits molecular mechanics. Development of parameters can be tedious, depending on the predictive accuracy required, but improvements in force fields continue (108–109). The increasing use of quantum mechanics (110) combined with analysis of experimental data (111) offers a strategy that should be generally used. Application to transition states has been attempted in the past (112, 113), but recent results by Houk (114) in which traditional methods are used to calculate the force field parameters for molecular mechanics offer considerable agreement with experimental observations. Weiner et al (115) studied trypsin hydrolysis by a hybrid approach.

Quantum Mechanics

The increasing computational power available makes the use of quantum mechanics more possible. Conjugated systems and transition states require consideration at this level. An increasing role as the basis for parameter determination for force fields is an obvious prediction. Clementi (116) and

Gresh & Pullman (117) developed parameters for larger molecular fragments such as amino acid residues by quantum methods and fit empirical functions to reproduce the energy surfaces. These energy functions are used to evaluate ion binding and hydration of nucleic acid oligomers (118) and other complex systems by Monte Carlo techniques. Questions concerning reactivity and mechanisms often require application of these techniques (119–121).

Molecular Dynamics

Molecular dynamics, a simulation technique, offers considerable insight into the vibrational modes accompanying transitions between conformational states. The computational requirements to simulate molecular behavior given the derivative of the potential energy field, the force, requires summation and mass weighting to determine the acceleration, which is added iteratively. The time steps necessary to simulate molecular motions are extremely small, femtoseconds, and the computational requirements to simulate phenomena of chemical and biological interest are enormous because of the large number of steps required. Another problem is the random walk of the procedure that occurs unless one is attempting to determine the energetics along a known transition path. Explicit inclusion of solvent offers an exciting approach to understanding solvation. The major caveat is the difficulty of ascertaining the extent of conformational space explored, the extreme computational demands, and the inherent limitations in current force field representations of electrostatics. Applications to numerous areas of chemistry are being reported (122–124) and are helping to establish confidence in this approach.

Monte Carlo Sampling

In the Monte Carlo sampling method, random conformations are generated and their energy is evaluated. By sufficient sampling, an overview of the energy surface is obtained and confidence limits may be placed on the relevance of the minimum energy conformation found to the global minimum. The Metropolis algorithm is normally used to reject conformers from the statistical sample whose energies are greater than would be predicted by the Boltzmann distribution (125–126). Paine & Scheraga (96) suggested a new approach. The application to the backbone conformation of the pentapeptide enkephalin produced results agreeing with those from energy minimization procedures.

Systematic Search

A systematic search is a uniform grid search of torsional space in which each conformation corresponding to a grid point is generated and its energy evaluated. The major advantage of this method is that all minima are located within the space examined and to the accuracy of the grid space. As this

procedure is combinatorial, its computational demands restrict its use to problems with limited torsional degrees of freedom (ten rotatable bonds) or in which constraints can be used to limit the search. The determination of the three-dimensional structure of the antibiotic aridicin A by Jeffs et al (105) combined constraints such as coupling constants and NOE measurements from NMR with systematic search to arrive at a unique structure. This method is basic to pharmacophore identification with the Active Analog Approach.

Distance Geometry

The distance geometry method is a mathematical transformation in which the geometrical constructs are expressed in terms of relative distances. One obvious advantage is direct comparison of objects without concern about rotational and translational transformations. This procedure has become an integral part of the Ghose & Crippen approach (76) to receptor site modeling, as well as to the determination of three-dimensional solution structure by NMR as exemplified by the procedure of Havel & Wuthrich (103). A novel and important application has been to pharmacophore identification by Sheridan et al (61). The major disadvantage is that the procedure as implemented relies on minimization, which identifies only the nearest solution and provides no information regarding the uniqueness of the solution. Crippen (127) and Purisima & Scheraga (128) suggested approaches to alleviating the local minima problem.

SUMMARY

Progress in genetic engineering has increased the need for, while advances in computational hardware have removed barriers impeding, the development of appropriate computational tools to assist in the understanding of molecular interactions. Advancements both in techniques and in broadening application have been clearly demonstrated. Further development requires progress in the fundamental aspects of theoretical chemistry as well as an increased base of experience in choosing the appropriate set of assumptions for a particular problem. Computer-aided drug design is a current reality, but one that, at its best, supplements an incomplete methodology with the traditional insight and wisdom of an experienced medicinal chemist. In the next few years progress in developing a sound theoretical foundation will make molecular design a realistic aid to the medicinal chemist and protein engineer.

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