Multi-author Review

Molecular recognition

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Molecular recognition – A universal molecular science?

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In a loose but non-trivial sense, every chemical phenomenon with the exception of unimolecular processes depends upon molecular, or atomic, recognition. Charges and dipoles direct the course of chemical reactions and influence the structures of liquids and solids. Non-polar and steric interactions added to these forces make up the basic mechanistic components of molecular recognition. Such a sweeping generalisation is more a curiosity than a stimulus for the field of science which molecular recognition has become. The current preoccupation in many countries with molecular recognition more significantly arises from a realisation of the importance of multipoint intermolecular interactions not only for the development of new chemistry, but also in applications in fields as diverse as molecular electronics and medicinal chemistry. In this connection, the interactions of chemistry and biology first discussed in the late 19th century are particularly significant.

Undoubtedly the roots of this science are in the work of Emil Fischer through his studies on asymmetric reactions of enzymes with carbohydrates[5]. His extensive work on the synthesis of monosaccharides provided Fischer with the first library of stereoisomeric compounds of known relative configuration and he was therefore uniquely well placed to investigate the reactions of glycosides with enzymes. The fact that enzymes showed selectivity with respect to only one of the possible isomers led Fischer to his famous lock and key hypothesis stated as follows:

'...the results suffice in principle to show that enzymes are choosy with respect to the configuration of their substrate, like yeast and other microorganisms. The analogy between both phenomena appears so complete in this respect that one may assume the same origin for them.... To use an image, I would say that the enzyme and glucoside must fit each other like a lock and key to be able to exert a chemical influence upon each other.... The facts proven for the complex enzymes will also soon be found with simpler asymmetric agents.'

Although now mechanistically inadequate for describing catalysis by enzymes, this hypothesis contained within it the germ of the concepts of molecular recognition. Contemporary with Fischer's studies, recognition phenomena, although not at this stage described in molecular terms, were being discovered with antibodies. Ehrlich had undertaken substantial work in the field of immunity and had redrawn Fischer's image in terms of immunology by describing the interaction as '...a key antigen in a lock antibody...'. Further in 1897, Ehrlich propounded a receptor theory of antibody synthesis suggesting that the binding of antigens to the side chains of surface membranes provoked new synthesis of these side chains[2]. Developments followed quickly for in 1900 Landsteiner identified human blood groups and natural isohaemagglutinins, the characteristic proteins of blood that group-specific antibodies recognise.

A generation later, the interaction of an antibody with a specific antigen was recognised experimentally by the precipitation of the complex and not through the occurrence of a chemical reaction. However, one of the first demonstrations of molecular recognition by antibodies was by Landsteiner and Scheer[9] who were able to show semi-quantitatively that the strength of binding of ortho-, meta-, and para- sulphonate-, carboxylate-, and arsonate-substituted anilines depended upon the position and nature of the acidic substituent. During this work, Landsteiner coined the word 'hapten' to describe groups that are not themselves capable of provoking antibody formation but can be recognised by antibodies[8]. Further, it gradually became clear that antibodies that interacted specifically with cell surfaces or with viruses, for example, could be isolated.

Related to the question of molecular recognition was the question of the mechanism of antibody formation, in other words how a vast number of chemically similar antibodies, serologically indistinguishable, but reacting with different antigens, can be produced in the same species. Many theories were propounded, all in the absence of experimental data on amino acid composition and protein structure. It was thought by Pauling, amongst others, that changes in the conformation of certain regions of the antibody were responsible for the multiplicity of recognition sites available to antibodies[8]. Although these ideas were inevitably sketchy, Pauling, in the mid-1940s, was able to give a remarkably clear defini-

tion of what we now understand as molecular recognition in terms of structure and bonding. He wrote in a special chapter in Landsteiner's book[8]: '...specificity can arise in the interaction of large molecules as a result of the spatial configuration of the molecules.... If.... the two molecules possessed such mutually complementary configurations that the surface of one conformed closely to the surface of the other there would be strong electronic van der Waals attraction between all of the atoms on the surface of one of the molecules and the juxtapposed atoms of the complementary surface of the other molecule.' Interestingly, the words 'complementary surface' were used unprompted by one of the authors of these reviews. To us today, the term 'electronic van der Waals attraction' is curious but Pauling clarified in discussion the various ionic, dipolar, and dispersion forces that would be involved. At this stage in the development of molecular recognition, metaphors were beginning to give way to mechanism as we understand it today.

In all fields of molecular recognition today, the emphasis is on a detailed study of the interactions between the partners, atom by atom if possible. For example, much of the work on antibodies before the 1940s was built upon the recognition of cell surface antigens. To bring the study of such interactions to a molecular level, it is necessary to be able to understand the structures of the cell surface molecules. Such an understanding has only recently become available. In the case of oligosaccharides, the work of Lemieux in Canada is outstanding[11]. As in the work of Fischer, the scientific understanding that was eventually obtained arose from an extensive exercise in selective chemical synthesis of oligosaccharides. This allowed Lemieux to realise that the recognition event between antibodies and oligosaccharides, for example, lectins, depends upon a primary hydrogen bonding interaction between hydroxyl groups of individual sugar residues reinforced by the glueing of hydrophobic patches (C–H bonds on the opposite face of the carbohydrate rings) through so-called hydrophobic bonding with the receptor. He was also able to identify the required conformations of the oligosaccharides by NMR spectroscopy thereby bringing modern stereochemical considerations into play. A further feature of general importance was the recognition of the role of the solvent, water, in such associations. The significance of desolvation in catalysis by enzymes and in molecular recognition in aqueous solution especially is a topic of current interest.

One of the practical aspects of molecular recognition of cell surface antigens, namely the production of vaccines, has been approached directly through concepts of molecular recognition. From a knowledge of the structure of an antigenic protein, small sequences (6–15 residues) can be selected as targets for the antibody to be present in the vaccine. As will be described later, a single antibody only recognises a small portion of the surface of a protein. Thus an antibody raised to the selected fragment should also bind to the intact antigen, provided that the conformation of the oligopeptide chosen is the same as the homologous sequence in the intact protein, a situation that will not always hold. Lerner has shown that this concept can be realised[12]. From the point of view of molecular recognition, it is a success, but from the point of view of useful vaccines, the technique sidesteps the normal stimuli to the mammalian immune system and hence the immunological memory will be lost. More frequent immunisation might then be necessary. Time will tell whether synthetic vaccines will have a significant future in medicine.

Illuminating and significant as these studies are, there can be no doubt that research on nucleic acids leading to the immense power of modern molecular biology has been the most directing influence in molecular recognition in the second half of this century. It would be an insult to readers of this journal to comment on the details of the Watson and Crick model of DNA structure centred upon base-pairing but the concept of a specific and stable interaction by hydrogen bonding between two heterocyclic bases has had at least as great an impact as had Fischer's ideas on the stereochemical control of enzyme-catalysed reactions. The selectivity of enzymes presents a striking challenge to chemists but such selectivity deals usually with a single chemical reaction. The fidelity of replication, transcription, and translation processes mediated by nucleic acids have added force in their challenge because they are at the very heart of the reproduction and maintenance of living organisms.

A chemist reacts instinctively to such a challenge from nature. Is this the only way to get such specific interactions in nucleic acids? Are there any other bases that might produce base pairs of similar strength to the naturally occurring ones? Are there other self-associating systems with the potential for self-replication? Can non-enzymic catalysts be designed that have similar selective and catalytic properties to those of enzymes? Can stereoselective synthesis be carried out with selectivity equal to that produced by enzymes? Many of these questions are not new and many of them can now be answered to a significant extent (see following articles by Rebek and myself). These big questions, and many subsidiary questions of detail have provided a remarkably strong driving force for advances in many fields of molecular science. Fischer himself attempted non-enzymic asymmetric synthesis as early as 1903[4]. It can be argued that recent advances in the synthesis of homochiral compounds by organometallic and other synthetic methodologies[16] have been a direct consequence of the challenge of enzymic selectivity to organic chemists.

Improvements in synthetic methodologies for the preparation of nucleosides and nucleotides have encouraged chemists recently to investigate the properties of analogues of nucleic acids. Two main approaches can be distinguished. On the one hand, variations in the structure of the heterocyclic bases can be used to investigate the formation and stability of non-natural base pairs in

a duplex structure and on the other hand, alternative backbones to ribose can be studied.

Both scientific curiosity and the possibility of discovering new useful classes of drugs stimulates this work. Tamm, for example, has made the surprising discovery that DNA polymerase can catalyse the formation of *pyridine*-pyrimidine base pairs. The results suggest that the less predominant tautomeric forms of pyrimidine bases in a template DNA strand can lead to mismatching of bases in the Watson-Crick sense and that such mispairs can elude the polymerase's proof reading activity[15]. Benner has also investigated novel bases[13] but has extended studies to examine analogues of ribose in the backbone[14]. One of the reasons for this investigation was the possibility that RNA analogues that were stable to chemical and enzymic degradation might have significance in the treatment of intractable human diseases. The instability of RNA is directly associated with the glycosidic linkage and the 2′-hydroxyl group. Stimulated by the success of acyclic nucleosides as anti-viral drugs, in which the drug molecules are recognised by the phosphorylating enzymes of a virally infected cell, Benner synthesised oligonucleotides in which the 2′ carbon atom was removed. Flexible nucleotide analogues resulted. Duplexes of the flexible nucleotides incorporated into ribonucleotides were obtained. However the results suggest that oligonucleotides of less than 15 bases composed entirely of flexible nucleosides will not form stable duplex structures with complementarity for natural oligonucleotides in aqueous solution. Further imaginative work will undoubtedly lead to a better understanding of how such oligomers recognise each other efficiently.

As has just been mentioned, one of the critical features of the biochemistry of nucleic acids is their capacity for self-assembly. Indeed one of the first strong modern bridges between chemistry and biochemistry was the recognition that biosynthetic pathways embody strongly features of the intrinsic chemical properties of the reactants usually enhanced by catalysis due to enzymes. The stereochemical course of such reactions has become a major field of interest for bioorganic chemists both at the level of whole organism and isolated enzyme studies and biosynthetic pointers direct naturally towards detailed molecular studies of key enzymes (see articles by Gani et al. and Robins). Chemists have always emphasised stereochemistry as an incisive probe of molecular recognition. Self-assembly itself has been invoked in fields other than nucleic acids by Eschenmoser with respect to the synthesis and biosynthesis of natural products[3]. The basis of his perception was the assembly of pyrrolic units in the biosynthesis of porphyrins and corrins leading to vitamin B_{12}. The inevitable chemical colonisation has also occurred as interests in the self-assembly of 'unnatural products' has developed[7]. Nobel Prize winning investigations of crown ether chemistry and its applications in molecular recognition and catalysis by Lehn[10] and Cram[1] serve to emphasise the growing significance of molecular topology as a fundamental field of investigation.

If that were all, it would be sufficient to sustain the interest of many scientists for many years. What makes molecular recognition special in today's scientific environment is its obvious relevance to many important applications in industry coupled with its intrinsic interest and challenges. Molecular recognition is therefore at once investigative, creative, and applied in character. In the applied sciences, studies in medicinal chemistry concerned with small molecule-receptor interactions have assumed special significance (see articles by Breckenridge and myself). The importance of intermolecular forces in the structure of materials has become a growth area[6]. Concepts of molecular recognition have revolutionised views on catalysis so that one can now adopt at least five experimental approaches to the design and discovery of catalysts based upon such ideas, namely biomimetic methods, chemically modified enzymes (see article by Wong), site-specific mutagenesis, catalytic antibodies (see article by Tedford and Stimson), and imprinting on polymers. With such breadth, it is impossible to cover its full range in a series of short review articles. I have therefore invited authors to contribute on topics that can be related clearly to antecedents in the original formative literature mentioned above to illustrate the investigative, creative, and applied aspects of the field.

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