DEVELOPMENTS IN COMBINATORIAL CHEMISTRY AND SELECTION APPROACHES TO CATALYTIC SYSTEMS

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SELECTION APPROACHES TO CATALYTIC SYSTEMS

Generation of a new catalyst is a daunting task for chemists, particularly mimicking the astonishing efficiency and selectivity of enzymatic catalysts so that these can be applied to important synthetic reactions. Synthesis of designed systems rarely lead to successful catalysts as the rules for conformation and intermolecular interactions are imperfectly understood and as a result, attempts to synthesize working catalysts by rational design of a particular structure fail. Focusing directly on finding systems that recognize transition states or their stable analogues provide large array of different molecules instead of a single structure being designed. From this pool of different catalysts, the best one is selected. This selection approach has increased the probability of success, since vastly more molecules are generated. While the idea is conceptually straightforward, putting the principles into practice has proved difficult. Herein, several 'selection' approaches in the generation of the system that can recognize transion state analogues are dealt with.

1. **Catalytic antibodies :** As a large part of the catalytic activity of enzymes result from stronger binding of the transition state than of the starting material(s), any molecule that can bind the transition state species selectively should be a catalyst for that reaction. With this in mind Jencks, in 1969, suggested that antibodies generated in the mammalian immune response should function as enzymes. Antibodies are proteins that are produced in the body in response to an alien species, called an antigen, and they bind to such a molecule or particle strongly and selectively.

2. **Ribozymes :** Ribozymes are RNA molecules which have some natural catalytic activity; for example *Tetrahymena* ribozyme can cleave certain RNA sequences with specificity similar to that of the RNA processing enzymes. The first example of an *in-vitro* evolved ribozyme was that demonstrated by Beaudry and Joyce. Starting from the *Tetrahymena* ribozyme which has a RNA cleavage activity, ribozyme with DNA cleavage activity was evolved, which accelerated the DNA cleavage reaction by a factor of 100 fold over the wild-type. Recently Bartel and Szostak screened a pool of polymerase ribozymes for the ability of a ribozyme to join a substrate to itself. Ribozymes that performed this reaction were successfully isolated from the pool by affinity column chromatography for the substrate and then amplified. Ribozymes clearly show potential in the field of catalysis, but it is likely that their structural make-up does not give enough diversity and specificity for them to rival peptide-based catalysts. Their evolutionary capacity should go some way to offset this deficiency if appropriate selection procedures for catalytic activity can be developed.

3. **Imprinted polymers :** Antibodies lack the features that are important for many practical applications, such as thermal stability and chemical robustness. They have a short lifetime and are expensive to produce and hence a synthetic analogue would be of much interest. Copying their mode of production synthetically is clearly not practical, but molecular-sized cavities can be generated in the solid state by polymerization in presence of a guest template. This is known as molecular imprinting or 'footprinting'. The process leading to an imprinted polymer is illustrated in the Fig. 1 : a bulk polymerizable monomer is mixed with a binding monomer and the guest molecule. Polymerization is then initiated with the polymer being formed around the imprint molecule. After removal of the guest, the polymer contains cavities of the correct size and shape for the imprint concerned.

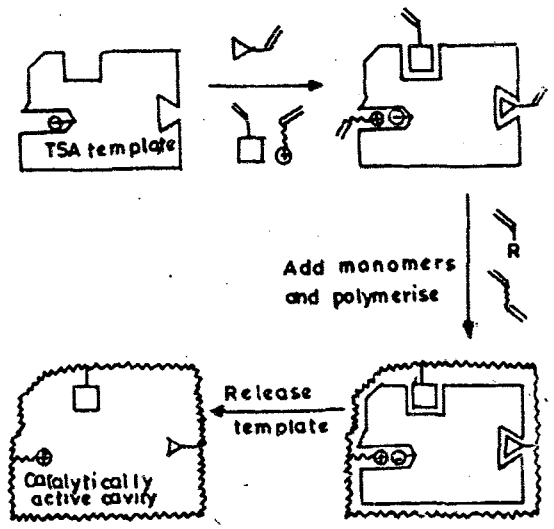

Fig.1.: Schematic representation of the process of imprinted polymer

An example of a self-assembly directed polymerization is the generation of a chiral stationary phase for separation of B-adrenergic blockers by polymerization of methacrylic acid and a crosslinker in the presence of a (S)-(-)-timolol template. The monomer forms noncovalent linkages (mainly hydrogen bonds) with the template in organic solution and this self-assembly holds the monomer units in appropriate positions during the polymerization process. The template is removed from the finished polymer by washing with acetic acid. The polymer generated in this way has a three-dimensional network, with template-complementary binding sites. When used in powder form as a stationary phase for chromatography, the polymer enables easy enantioselective separation of (S)-(-)- timolol from a racemic mixture.

Specifically imprinted polymers can now be prepared with some reliability. They are relatively simple and cheap to prepare, show good mechanical, chemical and thermal stability and can be stored for years without loss of activity.

4. **Thermodynamic templating :** Thermodynamic templating has been used for a variety of purposes but the concept as applied for generating the catalysts is shown pictorially in Fig. 2. If several building blocks were assembled in the presence of a guest, then at least one combination would be expected to bind the guest. This product should possess low energy relative to non-binding products and therefore be preferred if its assembly proceeds in a reversible and thermodynamically controlled fashion. Non-binding hosts produced should on average be proof-read and recycled into other products assuming that all possible hosts can be accessed without kinetic barriers. Any strongly binding product will become more concentrated in the reaction mixture through a process of thermodynamic templating and after isolation it could be identified as the best of all possible hosts. Furthermore, use of a different template should allow isolation of a different host from the same reaction mixture. These principles have been utilized in the generation of inorganic coordination complexes such as Lehn's self sorting helices, but as these compounds are held together only by relatively weak interactions in solution, they lack the robust character of covalent molecules.

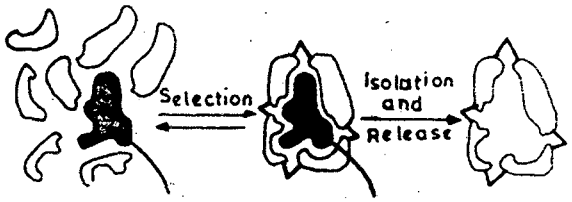

Fig. 2: Thermodynamic templating

Conclusion

Selection methods have made considerable progress and are now in regular use for lead generation. Each method has inherent problems: there is a numerical and analytical limit to the diversity accessible by library methods; imprinted polymers have heterogeneous and unknown binding sites; antibodies are expensive, inefficient, and their peptidic nature brings problems of thermal enzymic instability; and ribozymes, in addition to expense and instability, may lack the structural diversity necessary for effective host generation. The more recent idea of thermodynamic templating combines several of the best features of the other techniques but it too will require a wide range of building blocks in order to fulfil its potential.

Combinatorial Chemistry

Combinatorial chemistry is a term that describes a set of tools for generating vast chemical diversity rapidly and efficiently. The name 'combinatorial' stems from the fact that this technique is able to produce libraries of compounds in which every combination of the materials used will result in a new compound. The idea of combinatorial chemistry is to make a large number of chemical variants all at one time, to test them for bioactivity, binding with a target or other desired properties, and identify the most promising compounds for further development. Thus by employing building block collection and systematically assembling these blocks in many combinations using chemical, biological or biosynthetic procedures it is possible to create chemical libraries as vast populations

of molecules. Combinatorial Chemistry is thus an approach to produce molecular diversity synthetically.

Combinatorial synthesis

The idea of combinatorial synthesis is to form large libraries of molecules enmasse – instead of synthesizing the compounds one by one.

Methods

In combinatorial synthesis a range of monomers A_1 to A_n will react with a range of monomers $B_1 - B_n$ to produce possible n^2 compounds of the type $A_{1-n}B_{1-n}$.

For example, if an acid x and base y can react to give anamine and amide, the combinatorial synthesis can take a range of acids x_1 to x_n and bases y to y to give various combination of amides x y to x y.

Combinatorial compounds are created either by producing compounds bound covalently to "solid–phase particles" by producing "solution phase method". Sometimes biopolymers are also created on biological source.

Solid phase method

'Merrifield', in 1963 revolutionized the area of peptide synthesis by developing a new method of rapid synthesis: Solid–Phase synthesis. The solid phase technology used in a combinatorial library can be broken down into three major components:

1. first is the support that should be stable to a wide range of organic solvnents in reagents.
2. second is the 'linker', which connects the support to the scaffold or target molecules.
3. third is the 'target molecule' or 'scaffold' which should be synthesized in high yield purity.

Two different strategies in solid phase synthesis involve:

(1) Split and mix method : This method involves simultaneous creation of compounds and the mixture is then screened for performance. Reactions occur in both solid and solution phase and compounds are attached to the polymer beads. The resin is first divided into equal portions, treated with a reagent, washed, combined and mixed. This is spilled again and reacted with different reagents. After screening for a particular activity the most active compound is identified.

(2) Parallel synthesis : In parallel synthesis different compounds are synthesized in separate vessels (without re-mixing) often in an automated fashion. Unlike split synthesis, which requires a solid support, parallel synthesis can be done either on a solid support or in solution. A commonly used format for parallel synthesis is the 96-well micro titer plate. Robotic instrumentation is used to add different reagents to separate wells of a micro titer plate in a predefined manner to produce combinatorial libraries.

Solution phase synthesis

The drawbacks associated with solid phase synthesis can be overcome by solution phase synthesis with the exclusion of linkers and tagging. The products in solution are relatively easy to identify and characterized. Carell and co-workers have reported the use of a central core molecule with multiple reactive groups as a template for the construction of libraries. The essence of the principle is the use of either cubane or xanthene derivatives as tetra substituted core molecules, for library construction. A second strategy for the solution phase synthesis of libraries was reported by Cheng and co-workers: a compound with multiple reaction sites, N-tertiary butyloxy-carbonyl-iminodiacetic acid anhydride was used to prepare collections of small non- peptide organic molecules in solution by reaction with amines and carboxylic acid.

Biological source as a method

A variety of biological systems are available for library construction, phase particles, polysome plasmids, bacteria etc. However, choosing the appropriate system depends on the desirability of the features and characteristics of that particular type of library. Each of these methods employs the fusion of the 'guest' peptides to a cell surface protein of the host. A number of reports describe the use of maltose receptor *E. coli* (the outer membrane protein, *Lam B*) as a peptide fusion partner. Oligonucleotides have been inserted into plasmids encoding the *Lam B* gene to produce peptides fused into one of the extra cellular loops of the protein. These peptides are available for binding of antibodies and can elicit an immune response when the cells are administered to animals.

Computational chemistry in combinatorial chemistry

Computational tools for combinatorial chemistry includes molecular spreadsheet databases and software related to structure-activity relationship and drug design. Combinatorial libraries are usually represented by generic structures with small number of differing substituents (R); R-group contains alternate structures. Chemical designing also provides 3D Combinatorial software packages that allow mapping pharmacophore patterns of collections of compounds in a library. Several companies provide software connections from properties plot to a 3D pharmacomphore plot derived from the best hit.

Almost 10^{200} compounds of molecular weight less than 850 can be prepared. As it is not possible to deal with all of them best selection has to be done on the basis of liphophilicity, shape, branching, chemical functioning and specific binding features. Another related combinatorial approach involves combinatorial growth of molecules that are complementary to binding sites of an enzyme. Combinatorial Chemistry thus now involves many new challenges.

Enzymes in combinatorial chemistry

Enzymes have very high selectivity and activities under extremely mild conditions. They are used for asymmetric synthesis and for chiral multiplication.

Menger's Combinatorial Synthesis

According to Menger, the basic idea to catalyst design by combinatorial approach was to attach covalently onto polyallylamine, various combinations of eight-functionalised carboxylic acids via a one step amide formation as illustrated in Fig. 3. In addition, one of the three metals (Mg^{2+}, Zn^{2+}, Fe^{3+}) was complexed to the polymer, thus generating hundreds of potential polymeric catalysts, each differing in the nature and number of its functional groups. It was clear that certain combinations among the hundreds of investigated were far more effective than others.

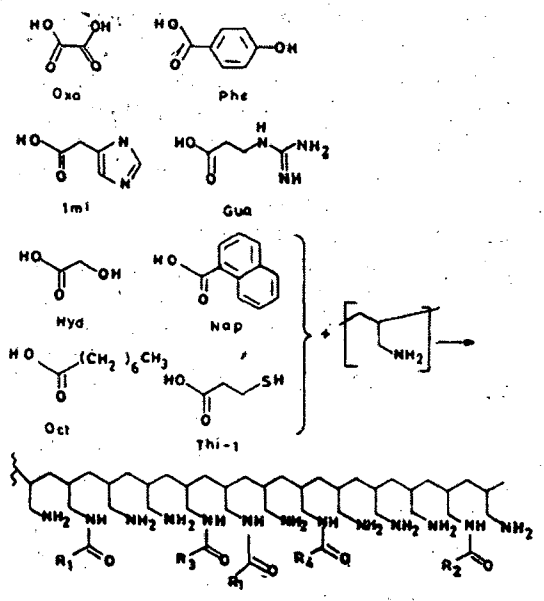

Fig. 3: Menger's combinatorial synthesis

Combinatorial chemistry applications

1. Semi-automated high throughput combinatorial solid phase synthesis : A semi-automated technique for massive parallel solid phase organic synthesis based on "split-only" strategy is described. Two different types of purpose-oriented reaction vessels are used. The initial steps are performed in domino, under resin bound intermediate, and then split into wells of micro plate for the last combinatory step. The domino block is a reaction block for manual and semi-automatic parallel solid phase organic synthesis that simplifies liquid exchange and integrates common synthesis steps. The synthesis in micro plate does not use any filter for separation of resin beads from the supernatant liquid and allows high throughput parallel synthesis.

This technique documented on example of disubstituted benzene includes the use of gaseous cleavage in the last synthesis step allows the synthesis of thousands of compounds per day in milligram quantities. It is based on:

1) Integration and automation of repetitive task.
2) Modular approach to use of dedicated tools and devices.
3) Split only technique and
4) Purpose oriented reaction vessels which include:

(a) The domino block for solid phase organic synthesis and

(b) Wells for micro plate, which allow handling of large number of compounds.

2. Synthesis of Functionalized aminodiol scaffolds : Combinatorial library motif has been developed based on orthogonally protected aminodiol scaffolds. Amine functionality is derivatized by commercially available electrophiles including carboxylic acids, sulfonyl chlorides, isocyanides and aldehydes. A hydroxyl moiety is converted to a carbonate linkage, allowing a variety of amines to be incorporated. The scaffold is anchored to a TentaGel at the second hydroxyl via a succinyl linker, which is hydrolyzed, by mild aqueous basic conditions. The method is used to make a library of about 17000 different members in mixture of five per sample.

3. Optimization of a HIV-1 TAT inhibitor by combinatorial lead structures : Lead molecules identified by combinatorial chemistry approaches are preferred starting point for straightforward improvement of compound profile. Structure guided rationals can be suppressed and complemented by systematic variation based on modular nature of the molecule.

A peptidic compound (CGP 64222), previously identified from a sequential unrandomization process was shown to specifically inhibit the interaction between the HIV-1 trans-activator Tat and its RNA responsible element TAR. Parallel synthesis with variations on one rationally defined position aimed at the identification of structural determination was undertaken to regain *in vitro* activity in biochemical and cellulose Tat-TAR interaction assays. As a result CGP74025 was identified; a drastically simplified but highly active TAT antagonist which is able to block HIV-1 replication even in primary human cells.

4. A model study with combinatorial libraries of tetraphenyl porphyrins asphotodynamic anti-cancer drugs : Tetraphenyl porphyrin libraries are developed via efficient combinatorial solution phase synthesis together with preliminary results from bioorganic study on their uptake in liposome membranes. Libraries with up to 666 components were prepared with substituents including Br, CF_3, Cl, CN, Me, Et, F, OAc, and Ph. Synthesis of more diverse libraries via a "latent libraries" approach is possible. This involves masking polar groups with lipophilic protecting groups. After purification of the latent library, the masking protecting groups are removed in a quantitative reaction that produces the library compounds as the only non-volatile components. Libraries can be characterized by laser desorption, mass spectrometry, NMR and UV-VIS spectoscopy. *In vitro* uptake into membranes of small sonicated liposomes can be measured both in terms of total porphyrin incorporation and in terms of structure incorporation relationship. Membrane incorporation has previously shown to correlate with photodynamic activity *in vitro* and *in vivo*. Therefore, these results may help to explain why photodynamic therapy of tumors, a modern anti-cancer treatment modality, is successfully performed with a complex mixture of porphyrins.

5. Combinatorial chemistry provides fresh and promising leads for medicinal chemistry : The recent emergence of combinatorial chemistry has greatly advanced the development of active lead compounds likes antineoplastic compounds, antimicrobial compounds, antiepileptic drugs. Benzodiazepine derivatives having different biological activities and many other important leads are getting discovered through tailoring combinatorial libraries.

Eg. benzodiazepines although are potent psychotherapeutic drugs, through combinatorial synthesis and high throughput screening, the derivatives also showed some other important biological activities. One is a benzodiazepine derivative that inhibits the interaction of antibodies with single stranded DNA – a process involved in systemic lupus erythematosus and used clinically as HIV antagonist. Another benzodiazepine derivative exhibits good opioid receptor antagonistic properties.

6. Applications of antibodies libraries obtained by combinatorial chemistry : The development of antibody combinatorial libraries have been applied to prevention or treatment of a series of human viral diseases such as HIV-1, respiratory syncytical virus (RSV) infection and Herpes simplex virus 1 and 2 infections. Additional applications are focused on obtaining a humanized antibody *in vitro* by resembling *in vivo* maturation. It can also be applied to obtain the antagonist or agonist or receptors involved in signal transduction and further facilitate the discovery of strategies to control signal transduction pathways.

7. Combining structure–based drug design and combinatorial chemistry for rapid lead-discovery : This method describes 'PROSELECT' which combines elements of structure based drug design and combinatorial chemistry to create a new paradigm for accelerated lead discovery. Starting with a synthetically accessible template positioned in the active site of the target of interest, 'PROSELECT', employs database searching to generate lists of potential substituents for each substituent positioned on the template. These substituents are selected on the basis of their being able to couple to the template using known synthetic routes and their possession of the correct functionality to interact with specified residues in the active site. The lists of potential substituents are then screened computationally against the active site using rapid algorithms. An empirical scoring function, correlated to binding free energy is used to rank the substituents art each position. The highest scoring substituents at each position can then be examined using a variety of techniques and a final selection is made. Combinatorial enumeration of the final lists generates a library synthetically accessible molecule, which may then be prioritized for synthesis and assay.

Analytical methods in combinatorial chemistry

1. Combinatorial library phase synthesis by capillary electrophoresis : Capillary electrophoresis monitors model reactions in combinatorial chemistry, especially for simultaneous alkylation reactions of secondary amines with a series of benzyl halides.

Capillary electrophoresis is used to monitor reactant and product concentrations in non-aqueous buffers. It is a useful tool for monitoring reactions to determine the initial rates, rate constants for quantitative analysis and is applicable for a variety of organic and bio-organic transformations. Capillary electrophoresis which can be used for a variety of solvents and wide pH range is a new and useful tool in optimizing product yields in solution phase reactions.

2. Use of optical spectroscopy in combinatorial chemistry

a) IR spectroscopy : IR spectroscopy is a general analytical method for resin samples. Internal reflections spectroscopy is especially suited for solid polymer substrate known as "pins" organic "crowns". Single bead analysis is done best by IR microspectroscopy, which allows for totally nondestructive analysis of resin samples. With automated accessories, diffuse reflection spectroscopy provides a method of high throughput on-bead monitoring of solid-phase reactions providing identification based on molecular structure. HPLC-FTIR is complimentary to LC-MS.

b) Raman spectroscopy : Raman spectroscopy as a complement to IR spectroscopy can be applied to resin samples and using RAMAN microscope to single beads.

c) Flurometry : Flurometry is an extremely sensitive detection method and allows rapid quantification of organic reaction directly on the resin.

3) Material development

DRAM (Dynamic Random Access Memory) computer chips with more than 30 combinatorial library and 4000 compounds in each, has been developed by Lucent Technologist USA. High super conductance by depositing combinatorial arrays of inorganic salts to create thin films of potentially super conducting composites are developed.

Future Directions and Prospects in Combinatorial Chemistry

Combinatorial chemistry has come a long way in the past few years, but many challenges still lie ahead. The development of solid-support chemistry, including new linkage supports, strategies and novel method for synthesizing support bound libraries and cleaving compounds from support are required. And combinatorics will continue to focus on different types of templates–novel structure templates for the versatile display of functionality. There are some interesting opportunities in the area of combining combinatorial strategies with computational strategies and structure based design. The idea is to use information about 3-D structures of receptors and enzymes in combination with libraries to rapidly identify high affinity ligands.

In the coming years, cloning and sequencing of the human genome promises that an unprecedented abundance of newly discovered proteins will become available as potential drug targets. Combinatorial methods will be called upon to provide such molecules to quickly and cheaply drive target validation. In this manner, the identification of leads will benefit from a significant, hidden benefit, which emerges from combinatorial screening. Hits derived from chemical libraries should be readily amenable to combinatorial analoging.

Conclusion

Combinatorial chemistry is a bit like a shotgun approach to drug discovery. Many 'start-up' and smaller companies have pioneered this technology, which is now being avidly taken up by the larger pharmaceutical companies. Combinatorial chemistry is likely to hinge on designing libraries in more rational ways. Although it is early in the game to predict just how important combinatorial chemistry will be for the future of pharmaceutical industries, it seems clear that combinatorial chemistry is rapidly becoming a significant new weapon in the arsenal of drug discovery chemist. Combinatorial chemistry should be considered as an advanced tool to aid in the rapid discovery of molecules with desired properties.

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