News Items

Thermostable Enzymes Produce Cyclodextrins*

Scientists at a small Canadian biotechnology firm have isolated two new enzymes that exhibit high thermostability and high productivity of cyclodextrins (CDs) from starch. Hiroyuki Aoki, Dennis Yao, Ernest Yu, and Masanaru Misawa of Allelix Inc., Mississauga, Ont., have recovered from soil bacteria found in Ontario two cyclodextrin glycosyltransferases (CGTases) – one yielding mostly α-CD, the other mainly β-CD.

Cyclodextrins are cyclic oligosaccharides, composed of six (α), seven (β), or eight (γ) glucose residues bound through an α-1,4 linkage. β-CDs are the least expensive and the most used commercially at present. α-CDs cost about 10 times as much, and γ-CDs 100 times as much. CDs are currently prepared by action of bacterial CGTases on gelatinized starch.

The torus configuration of CDs provides a hydrophobic cavity, enabling CDs to form inclusion compounds with a wide variety of materials. Commercial interest is growing in using CDs for separations and extractions, drug delivery, stabilizing agents, and other encapsulation applications in the food, pharmaceutical, and agrochemical industries. Allelix projects $50 million annual CD sales in the U.S. within a couple of years, with at least twice that if food uses are approved by the Food & Drug Administration. (Japan and several European nations already permit use in food.)

Allelix was founded in 1983 by Canadian Development Corp., a government corporation, which has supplied half the firm's funding. The province of Ontario has given 30%, and Canada's Labatt Brewing Co. 20%. Aoki described the group's work at the American Chemical Society's national meeting in Denver in April 1987.

One of the two CGTases isolated by the Allelix scientists produces primarily α-CD from starch, with high efficiency, and with high thermostability without adding calcium ions. The firm has not yet applied for patent protection on this enzyme and is keeping information about it proprietary for now.

The firm has just filed for patent protection in the U.S. on the other new CGTase. This enzyme produces mainly β-CD from potato or corn starch, with a beta-to-alpha ratio of as much as 5 : 1, depending on conditions. Total yield of CDs from 15% potato starch is 50% more than for a CGTase now used commercially, from *Bacillus macerans*. Moreover, unlike other CGTases, the new enzyme does not require prehydrolysis of starch by acid or amylase enzyme. Indeed, it performs better without prehydrolysis.

This new CGTase is secreted extracellularly by a bacterium assigned to the genus Bacillus. The bacterium may be a strain of *B. licheniformis*, but the Allelix scientists leave open the possibility it is a new species. This CGTase shows activity over a wide range of pH, with an optimum at 5.5. Optimum temperature for activity is 65 to 70 °C, important to enable rapid industrial-scale production of CD. (CGTase from

B. macerans is inactivated at 55 °C or higher.) The enzyme shows high thermostability without adding calcium ions, but as with most CGTases, calcium improves thermostability. However, other divalent cations – such as manganese, magnesium, and cobalt – also exert stabilizing effects, something not found with other CGTases.

The enzyme contains about 680 amino acid residues per molecule. It appears to be a dimer of identical subunits, each weighing about 72 000 daltons. By contrast, the CGTase from *B. macerans* is monomeric in structure, with a molecular weight of about 70 000 daltons.

Comparison of amino acid compositions reveals that the new enzyme has lower contents of valine and isoleucine, and higher levels of threonine, asparagine, and cysteine than does *B. macerans*. The amino acid sequence of 29 *N*-terminal residues shows about 70% homology between the new enzyme and CGTase from *B. macerans*.

Most notably, the new CGTase apparently contains two cysteine residues per subunit. These cysteine residues probably form disulfide bonds, contributing to the greater thermostability shown, compared with other CGTases.

Purification of the new enzyme by DEAE-Zetaprep chromatography is a relatively simple and effective procedure, because it is readily adsorbed to starch. A single step yields CGTase of sufficient purity for crystallization and amino acid sequence analysis.

The firm is now scaling up to pilot-plant production (using a 400-L fermentor). And several starch producers are evaluating CGTase samples. If the evaluations are favorable, Aoki says, Allelix will seek joint ventures to produce CDs, rather than make CDs itself.

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Information

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The Royal Swedish Academy of Sciences has decided to award the 1987 Nobel Prize in Chemistry jointly to Professor Donald J. Cram, University of California, Los Angeles, USA, to Professor Jean-Marie Lehn, Université Louis Pasteur, Strasbourg, and Collège de France, Paris, France, and to former research chemist Charles J. Pedersen, Du Pont, Wilmington, Delaware, USA for their development and use of molecules with structure-specific interactions of high selectivity.

Awarded for syntheses of molecules that mimic important biological processes

Summary

This year's Nobel Prize in Chemistry has been awarded to **Donald J. Cram**, USA, **Jean-Marie Lehn**, France and **Charles J. Pedersen**, USA for their development and application of molecules with highly selective structure-specific interaction, i.e. molecules that can "recognize" each other and choose with which other molecules they will form complexes. The laureates have been rewarded for synthesising organic compounds of low molecular weight and with very special properties. The molecules in these compounds are designed principally to bind cations (positive ions), but also anions (negative ions) and neutral molecules, in a specific and selective manner. The three researchers have studied chemical and physical properties of these complexes and have elucidated the factors that determine the ability of the molecules to regocnise each other and fit into one another like a key fits a lock.

Molecules have been produced that mimic the mode of action of enzymes. The laureates' research has been of great importance for developments within coordination chemistry, organic synthesis, analytical chemistry and bioorganic and bioinorganic chemistry, and has thus laid the foundation for the active interdisciplinary area of research within chemistry that has now come to be termed host-guest chemistry or supramolecular chemistry.

KUNGL. VETENSKAPSAKADEMIEN THE ROYAL SWEDISH ACADEMY OF SCIENCES 14 October 1987

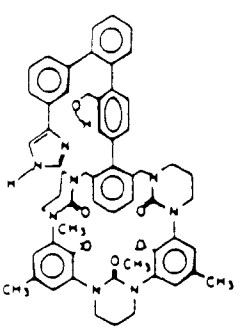
Cram in particular, using advanced organic-sythetic engineering and molecular mechanics calculations, has designed completely immobile host molecules that form particularly strong complexes of extremely high selectivity. Thus, for example, a host molecule with a 420 000-times-stronger tendency to bind sodium ions than to bind lithium ions has been synthesised. In addition to these alkali ions and other metal ions, it has been possible to produce host molecules that bind organic positive ions (cations such as diazonium and alkylammonium ions), as well as other host molecules which can bind small neutral molecules or negative ions (anions such as phosphate ions and organic carboxylate). Through their detailed investigations of the structures, physical properties and chemical reactions of the complexes, Lehn and Cram have increased our understanding of the factors determining the structure-specific interaction of high selectivity.

These examinations have also contributed to our understanding of ion transport via biological membranes. Selective cation binding has already found many applications. Using different types of host molecule it is possible, for example, to extract radioactive strontium or toxic cadmium and lead ions without affecting other ions, which is very interesting in terms of protection of the environment. Such high selectivity has been achieved that it is even possible to separate isotopes of the same element. Within analytical chemistry, selective complex formation has led to the development of ion-selective electrodes and other types of cation sensor. Certain transition metal complexes also show catalytic activity in photochemical processes, for example the photochemical decomposition of water to hydrogen, which may be of significance in energy production.

This formation of complexes is also being applied increasingly in organic synthesis, not least through Cram's success in producing crown ethers that help in separating the mirror images of aminoacids.

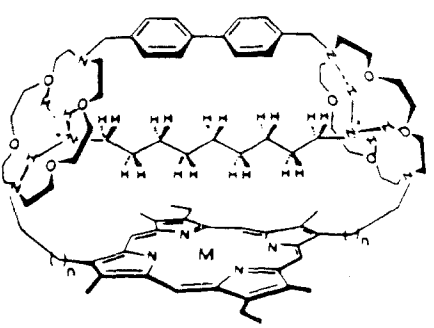
The goal is to produce synthetic host molecules that recognize biologically active molecules. Thus Lehn has produced a host molecule for the signal substance acetylcholine, which is so important in humans and animals.

The explosive development of the art of organic synthesis has enabled Cram and Lehn to produce hosts that to some extent mimic enzymes such as proteases, ATP-ases and transacylases (see Fig. 2). Supercomplexes which bind organic substrates and metal ions have recently been produced by Lehn (see Fig. 3). It will thus be possible to produce supermolecules which do not suffer from the present limitations on substrate structure and reaction type in, for example, enzymes. Through their work, Cram, Lehn and Pedersen have shown the way.



Molecule that partly mimics an enzyme (transacylase) according to Cram

Figure 2



Supercomplex according to Lehn

Figure 3

Background Information

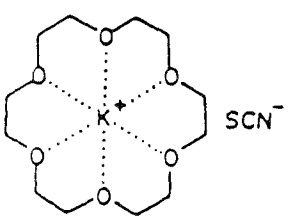
At the basis of many biological processes lies the ability of molecules to recognize each other and to form well-defined complexes. Well-known examples are substrates bound to enzymes, signal substances bound to receptors, antibodies bound to antigens and metal ions bound to ionophores. In most cases, one or more compounds of low molecular weight bind to a specific region in a high-molecular-weight compound, most often a protein or a nucleic acid. The binding is very specific and selective and the low-molecular-weight compound must fit the high like a key in a lock.

Inorganic chemists have long dreamed of sythesising relatively uncomplicated organic compounds that perform the same functions as natural proteins. Great progress towards this goal has been made over the last 20 years, and it is the pioneering achievements in this particular area that are now being recognized.

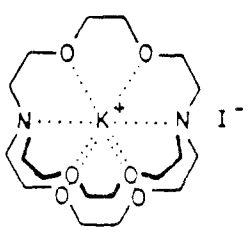
In 1967, Charles J. Pedersen published two works, which have now become classics, describing methods of synthesising cyclic polyethers, which he named crown ethers. Pedersen showed that these compounds have remarkable and unexpected properties and that they can even bind the alkali metal ions of lithium, sodium, potassium, rubidium and caesium into complexes in which the lithium ion is the smallest and the caesium ion the largest. He also found that, depending on the structure of the crown ehter, potassium could for instance be bound before caesium. Simply expressed, the selectivity is determined by the fact that different crown ehters include "holes" of different sizes, into which different spherical metal ions fit.

By building on Pedersen's fundamental discovery, Jean-Marie Lehn in 1969 developed bycyclic compounds of crown ehter type which he called cryptands and which show even higher selectivity when forming complexes.

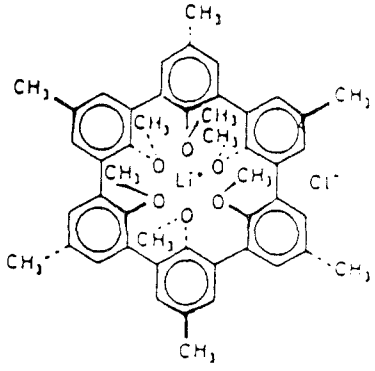
Jean-Marie Lehn and Donald J. Cram have subsequently each developed increasingly sophisticated organic compounds which when forming complexes leave fissures and cavities where low-molecular-weight compounds with different types of geometry can be bound. With this work, Pedersen, Lehn and Cram laid the foundations of what is today one of the most active and expanding fields of chemical research, a field for which Cram has coined the term host-guest chemistry while Lehn calls it supramolecular chemistry.



Crown ether complex according to Pedersen



cryptand complex = cryptate according to Lehn



host-guest complex according to Cram

Figure 1

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