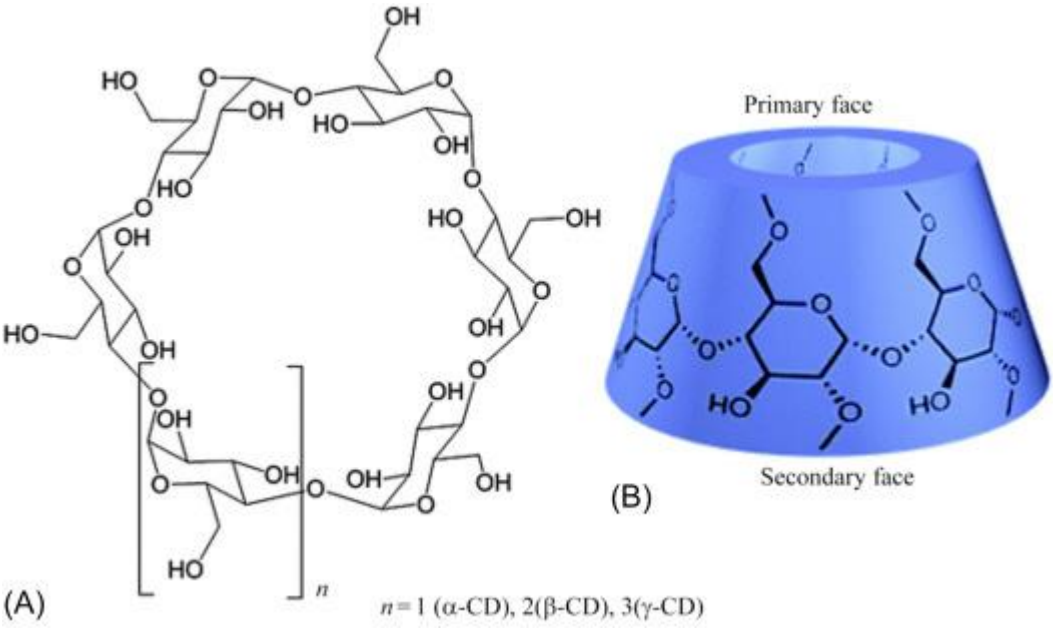
Unit-02: Bioorganic and Supramolecular Chemistry-II

Cyclodextrins: Structure, property, applications. Enzymes: enzyme kinetics, mechanism; application of enzymes in organic synthesis, model enzymes based on cyclodextrins.



Cyclodextrins are a family of cyclic oligosaccharides, consisting of a macrocyclic ring of glucose subunits joined by α-1,4 glycosidic bonds. Cyclodextrins are produced from starch by enzymatic conversion. They are used in food, pharmaceutical, drug delivery, and chemical industries, as well as agriculture and environmental engineering.

Cyclodextrins are composed of 5 or more α-D-glucopyranoside units linked 1->4, as in amylose (a fragment of starch). Typical cyclodextrins contain a number of glucose monomers ranging from six to eight units in a ring, creating a cone shape:

- **α (alpha)-cyclodextrin**: 6 glucose subunits
- **β (beta)-cyclodextrin**: 7 glucose subunits
- **γ (gamma)-cyclodextrin**: 8 glucose subunits

Synthesis

Cyclodextrins are prepared by enzymatic treatment of starch. Commonly cyclodextrin glycosyltransferase (CGTase) is employed along with α-amylase. First starch is liquified either by heat treatment or using α-amylase, then CGTase is added for the enzymatic conversion. CGTases produce mixtures of cyclodextrins, thus the product of the conversion results in a

mixture of the three main types of cyclic molecules, in ratios that are strictly dependent on the enzyme used: each CGTase has its own characteristic α:β:γ synthesis ratio. Purification of the three types of cyclodextrins takes advantage of the different water solubility of the molecules: β-CD which is poorly water-soluble (18.5 g/L or 16.3mM) (at 25^0C) can be easily retrieved through crystallization while the more soluble α- and γ-CDs (145 and 232 g/L respectively) are usually purified by means of expensive and time consuming chromatography techniques. As an alternative a "complexing agent" can be added during the enzymatic conversion step: such agents (usually organic solvents like toluene, acetone or ethanol) form a complex with the desired cyclodextrin which subsequently precipitates. The complex formation drives the conversion of starch towards the synthesis of the precipitated cyclodextrin, thus enriching its content in the final mixture of products. Wacker Chemie AG uses dedicated enzymes, that can produce alpha-, beta- or gamma-cyclodextrin specifically. This is very valuable especially for the food industry, as only alpha- and gamma-cyclodextrin can be consumed without a daily intake limit.

Structure

Typical cyclodextrins are constituted by 6-8 glucopyranoside units. These subunits are linked by 1,4 glycosidic bonds. The cyclodextrins have toroidal shapes, with the larger and the smaller openings of the toroid exposing to the solvent secondary and primary hydroxyl groups respectively. Because of this arrangement, the interior of the toroids is not hydrophobic, but considerably less hydrophilic than the aqueous environment and thus able to host other hydrophobic molecules. In contrast, the exterior is sufficiently hydrophilic to impart cyclodextrins (or their complexes) water solubility. They are not soluble in typical organic solvents.

Applications

Drug delivery

Cyclodextrins are ingredients in more than 30 different approved medicines. With a hydrophobic interior and hydrophilic exterior, cyclodextrins form complexes with hydrophobic compounds. Alpha-, beta-, and gamma-cyclodextrin are all generally recognized as safe by the U.S. FDA. They have been applied for delivery of a variety of drugs, including hydrocortisone, prostaglandin, nitroglycerin, itraconazol, chloramphenicol. The cyclodextrin confers solubility and stability to these drugs. The inclusion compounds of cyclodextrins with hydrophobic molecules are able to penetrate body tissues, these can be used to release biologically active compounds under specific conditions. In most cases the mechanism of controlled degradation of such complexes is based on pH change of water solutions, leading to the loss of hydrogen or ionic bonds between the host and the guest molecules. Alternative means for the disruption of the complexes take advantage of heating or action of enzymes able to cleave α-1,4 linkages between glucose monomers. Cyclodextrins were also shown to enhance mucosal penetration of drugs.

Chromatography

β-cyclodextrins are used to produce stationary phase media for HPLC separations.

Enzymes are proteins that act as biological catalysts by accelerating chemical reactions. The molecules upon which enzymes may act are called substrates, and the enzyme converts the substrates into different molecules known as products. Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life.

Enzymes are known to catalyze more than 5,000 biochemical reaction types. Other biocatalysts are catalytic RNA molecules, called ribozymes. Enzymes' specificity comes from their unique three-dimensional structures.