ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/268983067

γ-Cyclodextrin a review on enzymatic production and applications

DATASET · DECEMBER 2014

CITATIONS
11

READS
112

7 AUTHORS, INCLUDING:

Miao Wang
Chinese Center For Disease Control And Pre…
70 PUBLICATIONS **750** CITATIONS
SEE PROFILE

Feng Wang
Jiangnan University
16 PUBLICATIONS **160** CITATIONS
SEE PROFILE

Jian Chen
Nanjing University
637 PUBLICATIONS **6,103** CITATIONS
SEE PROFILE

All in-text references underlined in blue are linked to publications on ResearchGate, letting you access and read them immediately.

Available from: Zhaofeng Li
Retrieved on: 24 January 2016

Appl Microbiol Biotechnol (2007) 77:245–255
DOI 10.1007/s00253-007-1166-7

MINI-REVIEW

γ-Cyclodextrin: a review on enzymatic production and applications

Zhaofeng Li · Miao Wang · Feng Wang · Zhengbiao Gu · Guocheng Du · Jing Wu · Jian Chen

Received: 10 July 2007 / Revised: 13 August 2007 / Accepted: 15 August 2007 / Published online: 22 September 2007
© Springer-Verlag 2007

Abstract Cyclodextrins are cyclic α-1,4-glucans that are produced from starch or starch derivates using cyclodextrin glycosyltransferase (CGTase). The most common forms are α-, β-, and γ-cyclodextrins. This mini-review focuses on the enzymatic production, unique properties, and applications of γ-cyclodextrin as well as its difference with α- and β-cyclodextrins. As all known wild-type CGTases produce a mixture of α-, β-, and γ-cyclodextrins, the obtaining of a CGTase predominantly producing γ-cyclodextrin is discussed. Recently, more economic production processes for γ-cyclodextrin have been developed using improved γ-CGTases and appropriate complexing agents. Compared with α- and β-cyclodextrins, γ-cyclodextrin has a larger internal cavity, higher water solubility, and more bioavailability, so it has wider applications in many industries, especially in the food and pharmaceutical industries.

Keywords Gamma-Cyclodextrin · Cyclodextrin glycosyltransferase · Enzymatic production · Application · Property

J. Wu (✉) · J. Chen (✉)
State Key Laboratory of Food Science and Technology,
Jiangnan University,
1800 Lihu Ave.,
Wuxi 214122, People's Republic of China
e-mail: jingwu@jiangnan.edu.cn
e-mail: jchen@jiangnan.edu.cn

J. Wu · J. Chen
School of Biotechnology, Jiangnan University,
1800 Lihu Ave.,
Wuxi 214122, People's Republic of China

Z. Li · F. Wang · G. Du
Key Laboratory of Industrial Biotechnology,
Ministry of Education, School of Biotechnology,
Jiangnan University,
1800 Lihu Ave.,
Wuxi 214122, People's Republic of China

M. Wang · Z. Gu
Key Laboratory of Food Science and Safety,
Ministry of Education, School of Food Science and Technology,
Jiangnan University,
1800 Lihu Ave.,
Wuxi 214122, People's Republic of China

Introduction

Cyclodextrins are cyclic α-1,4-glucans composed of six to more than 100 glucose units (Qi et al. 2007). The steric arrangement of glucose units in the cyclodextrin molecule results in the shape of a hollow truncated cone with a hydrophilic outside surface, which makes cyclodextrins water soluble, and a hydrophobic internal cavity, which enables cyclodextrins to form inclusion complexes with various hydrophobic guest molecules (van der Veen et al. 2000a). The advantageous changes of guest molecular properties after the formation of inclusions complexes with cyclodextrins have led to many applications of cyclodextrins in the industries related to food, pharmaceuticals, cosmetics, chemicals, agriculture, etc. (Martin Del Valle 2004; Szente and Szejtli 2004).

Cyclodextrins are produced from starch or starch derivates using cyclodextrin glycosyltransterase (CGTase, EC 2.4.1.19). The enzymatic product is usually a mixture of cyclodextrins, including mainly α-, β-, and γ-cyclodextrin consisting of six, seven, or eight glucose units, respectively (Fig. 1), and trace amounts of large-ring cyclodextrins with more than nine glucose units (Terada et al. 1997). Although, during the past decade, a few interesting large-ring cyclodextrins showing novel structural features have been isolated and characterized (Endo et al. 2002; Zheng

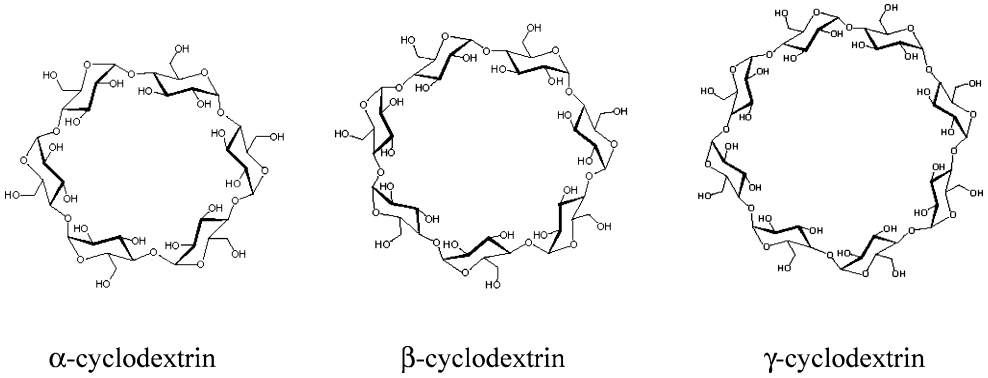

Fig. 1 Chemical structures of α-, β- and γ-cyclodextrins

et al. 2002; Qi et al. 2007), α-, β-, and γ-cyclodextrins are the most extensively studied and utilized products (Szejtli 1998).

Compared with α- and β-cyclodextrins, γ-cyclodextrin exhibits more favorable properties in terms of the size of its internal cavity, water solubility, and bioavailability, so it has even wider applications in many fields, especially in the food and pharmaceutical industries. However, to date, the most marketed cyclodextrin is β-cyclodextrin and to lesser extent α-cyclodextrin, while the market share of γ-cyclodextrin is considerably small because of its low yield and high price (Szejtli 2004). At present, the study of γ-cyclodextrin is booming, and the group of scientists interested in γ-cyclodextrin is continually growing (Takada et al. 2003a, b; Hirano et al. 2005; Nakagawa et al. 2006). Many attempts have been made to improve the production processes and to modify the properties of CGTases to enhance the yield of γ-cyclodextrin. It is expected that, with the price coming down, the market share of γ-cyclodextrin will increase significantly in the next decade (Biwer et al. 2002).

After the first fundamental review on cyclodextrins published in 1957 (French 1957), a few other excellent reviews and monographs have become available, which give some critical compilation of cyclodextrin-related literature to readers. The majority of previous reviews focused on CGTases and/or all three cyclodextrins (Hedges 1998; Buschmann and Schollmeyer 2002; Biwer et al. 2002; Martin Del Valle 2004; Szejtli 1998, 2003, 2004; Szente and Szejtli 2004). The present mini-review is dedicated to the enzymatic production, unique properties, and applications of γ-cyclodextrin as well as its difference with α- and β-cyclodextrins.

γ-CGTase

CGTase is a unique enzyme capable of converting starch or starch derivates into cyclodextrins via the cyclization reaction (Fig. 2), which is the basis of its industrial application. Besides cyclization, CGTase also catalyzes three other reactions: a coupling reaction, a disproportionation reaction, and a weak starch hydrolyzing reaction (van der Veen et al. 2000b). CGTase is an extracellular enzyme that is produced by a variety of microorganisms, including bacteria and archaea. The most extensively studied CGTases are from the *Bacillus* species (Tonkova 1998; Biwer et al. 2002). Because all known wild-type CGTases produce a mixture of α-, β-, and γ-cyclodextrins, they have been further classified into α-, β-, and γ-CGTases according to their major cyclodextrin products (Penninga et al. 1995). However, most CGTases studied so far are characterized as α- or β-CGTases and only few as γ-CGTase. Table 1 summarizes some γ-CGTase-producing bacteria sources and their enzymatic properties. The preparation of γ-CGTase is very similar to that of the other CGTases and has been described in detail elsewhere (Kato and Horikoshi 1986; Yang et al. 2001; Takada et al. 2003a; Wang et al. 2004, 2005, 2006a, b).

Because the separation of individual cyclodextrin from their mixture is costly and time consuming, the CGTases predominantly producing a single-type cyclodextrin are of interest (Martins and Hatii-Kaul 2002). Separation and purification of γ-cyclodextrin from cyclodextrin mixtures are particularly difficult because of its high water solubility, so the availability of γ-CGTases capable of producing an increased ratio of γ-cyclodextrin is desired. Several attempts have been made to screen γ-CGTase-producing microbial strains. Takada et al. (2003b) identified a novel alkalophilic bacterium, *Bacillus clarkii* 7364. γ-CGTase from this strain can convert 13.7% of pregelatinized potato starch into cyclodextrins, and γ-cyclodextrin reaches 79% of the product. Wang et al. (2004) screened a γ-CGTase-producing bacterium, identified as *Bacillus macorous*. The ratio of α-, β-, and γ-cyclodextrins produced by this CGTase is 2.5:1:7.2. More recently, Hirano et al. (2005) found a novel CGTase from alkalophilic *Bacillus* sp. G-825-6. This enzyme produces primarily γ-cyclodextrin and yields no α-cyclodextrin at any pH. Meanwhile, the ratios of γ-/β-cyclodextrin are always more than 1.7 and 4.7 with 1 and

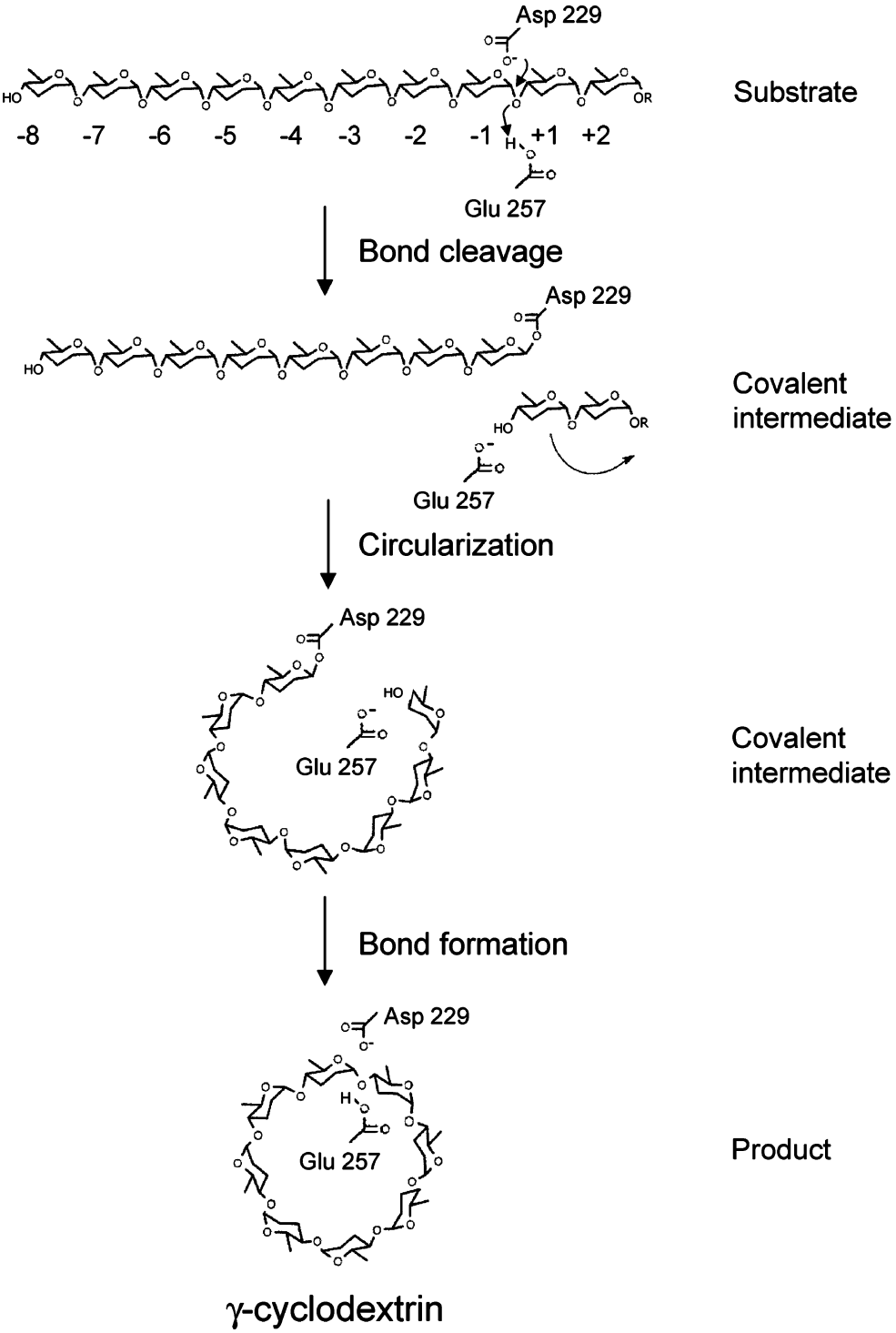

Fig. 2 Scheme of the γ-cyclization reaction of CGTase. The enzyme first cleaves the α-1,4-glycosidic bond between the residues bound at subsites +1 and −1, resulting in a covalent intermediate. The linear chain of the intermediate assumes a cyclic conformation, which is the circularization step. Subsequently, an α-1,4-glycosidic bond is reformed with the terminal 4-hydroxyl group of the intermediate. The catalytic residues involved in bond cleavage are Asp229 and Glu257 (numbering in *B. circulans* 251 CGTase), which are absolutely conserved in the α-amylase family (Uitdehaag et al. 2002b)

10% soluble starch as substrate, respectively. These new γ-CGTases are potentially useful in the industrial production of γ-cyclodextrin.

Besides screening of microbial strains, many efforts have been made to increase the yield of γ-cyclodextrin by changing the product specificity of CGTases through protein engineering. On the basis of structural analysis of CGTases (Lawson et al. 1994; Harata et al. 1996; Strokopytov et al. 1996; Uitdehaag et al. 1999a, b, 2000a), the active center of CGTase has been proposed to contain at least nine sugar-binding subsites designated as −7 through +2 (Qi and Zimmermann 2005). Sequence alignment and detailed analysis of CGTases indicate that there are clear differences between the γ-CGTases and the α-, β-CGTases in two regions that may be involved in the product specificity (Takada et al. 2003b). The first region (145–152; number-

Table 1 Sources and physicochemical properties of γ-CGTases

Bacteria species	Optimal ph	Optimal temperature (°C)	Molecular mass	Isoelectric point	pH stability	Thermal stability (°C)	Main product	Reference
Bacillus subtilis strain 313	8.0	65	64000	7.1	5.5~8.5	50	γ	Kato and Horikoshi (1986)
B. macorous strain WSH02–06	6.5[a]	50[a]	74,000[a]	4.96[a]	5~8[a]	40[a]	γ[b]	Wang et al. (2004)
Bacillus sp. strain AL-6	7.5~10.5	55	74,000	3~4	5~8	40	γ	Fugita et al. (1990)
Brevibacterium sp. strain 9605	10	45	75,000	2.8	6~8	50	γ	Mori et al. (1994)
Bacillus clarkii strain 7364	10.5~11	60	66,000	3.98	6~11	30	γ	Takada et al. (2003b)
Bacillus sp. strain G-825-6	8.0~10	55	78,200	-	7~12	50	γ	Hirano et al. (2005)
Bacillus sp. strain 32-3-10	8.0	50	-	-	-	60	γ/α	Yang et al. (2001)
Brevibacillus brevis strain CD162	8.0	55	75,000	6.3	5.5~9.0	50	γ/β	Kim et al. (1998)
Bacillus sp. strain 7-12	5.0, 8.5	60	69,000	-	6~10	70	γ/β	Cao et al. (2005)
Bacillus firmus strain 290-3	6~8	60	75,000	4.1	5~10	50	γ/β	Englbrecht et al. (1988)

"γ/α" and "γ/β" indicates that CGTase produces an approximately equal mixture of γ- and α-cyclodextrin, γ- and β-cyclodextrin, respectively.
[a] Unpublished data in our laboratory.
[b] From Wang et al. (2004)

ing in *B. circulans* 251 CGTase) has been identified at subsite −7, which is located on the starting loop of the B-domain. The amino acid sequences in this region of α- and β-CGTases are SSTDPSFA and SSDQPSFA, respectively. In contrast, there are only two amino acid residues (DI) in the region of γ-CGTases, suggesting that more space for the binding of glucosyl chain is required to achieve a higher level of γ-cyclization activity. Indeed, a mutant of the β-CGTase from *B. circulans* strain 8, in which residues 145–151 were replaced by a single aspartate residue, showed the enhanced γ-cyclodextrin production (Parsiegla et al. 1998). The second region has been identified at subsite −3, which is made up of the residues 47 and 87–94 (numbering in *B. circulans* 251 CGTase; Uitdehaag et al. 1999b). Residue 47 is a lysine and an arginine in α-CGTases and β-CGTases, respectively, but is replaced by a conserved threonine in γ-CGTases (van der Veen et al. 2000c). Furthermore, both α- and β-CGTases have the sequence INYSGVN(N) at loop 87–93 and 94, while the stretch sequence of HP–GGF– is found in γ-CGTases, which results in shorter loops in γ-CGTases than those in α- and β-CGTases. This difference could also suggest that more space for the binding of glycosyl chain is needed for a higher level of γ-cyclization activity. These detailed insights allow the rational construction of mutant γ-CGTases with desired γ-cyclization activity.

Enzymatic production of γ-cyclodextrin

The amount of cyclodextrins and the ratio of α-, β- and γ-forms in the product are determined not only by the CGTase but also by the reaction conditions, including reaction time, temperature, and presence of solvent (Goel and Nene 1995). In general, two types of cyclodextrin production processes are used (Schmid 1996). One is the solvent process, which requires an organic complexing agent to extract one type of cyclodextrin selectively and thus directs the enzymatic reaction to produce the cyclodextrin of interest; the other is the nonsolvent process, which does not require complexing agents and produces a cyclodextrin mixture that can be further separated by chromatographic procedures. On an industrial scale, γ-cyclodextrin is produced using the solvent process.

During the enzymatic reaction, the accumulation of γ-cyclodextrin inhibits its own synthesis and favors the formation of other cyclodextrins, whereas the extraction of γ-cyclodextrin with a solvent-complexing agent can reduce its concentration in the water solution and consequently decrease product inhibition. Consequently, the yield and selectivity of γ-cyclodextrin are significantly influenced by the use of appropriate complexing agents, which form insoluble or highly stable inclusion compounds with

γ-cyclodextrin. For example, Bender (1983) employed a combination of bromobenzene and sodium acetate as complexing agents to obtain γ-cyclodextrin with a yield of 18.7%. Sato and Yagi (1991) obtained γ-cyclodextrin with a yield of 40% by using pentacyclic and tetracyclic terpenoids (such as glycyrrhizic acid and stevioside). Matioli et al. (2000) have used glycyrrhizin to increase the yield of γ-cyclodextrin. However, these complexing agents are not ideal for industrial production because of their low selectivity for γ-cyclodextrin. Rendleman (1992, 1993), Schmid (1996), and Shieh (1996) found that some cyclic complexing agents with 12–24 atom rings, such as cyclododecanone and 8-cyclohexadecen-1-one, can be used to effectively enhance the yield and selectivity of γ-cyclodextrin. Currently, cyclic compounds are widely used in the enzymatic production of γ-cyclodextrin. However, their application also has several disadvantages in the aspect of toxicity, flammability, expensiveness, and the need for a solvent recovery process (van der Veen et al. 2000a). Consequently, the availability of γ-cyclodextrins is still limited in spite of a great market demand.

A typical flow sheet of the solvent process for γ-cyclodextrin production is shown in Fig. 3 (Schmid 1996). In the first step, the liquefaction of starch (typical at a starch concentration of 20–30%) is carried out using α-amylase, acid, or mechanical disintegration to make starch suitable for the incubation with CGTase at lower temperatures. On an industrial scale, liquefaction is usually achieved by α-amylase treatment and jet cooking. The liquefied starch is treated with CGTase under controlled pH and temperature. Cyclohexadecen-1-one or other appropriate complexing agents is added to extract the formed γ-cyclodextrin. After the enzymatic reaction, the complex of γ-cyclodextrin/complexing agent and excess complexing agent are separated from the reaction solution by centrifugation. The supernatant contains unused starch, maltodextrin, glucose, maltose, CGTase, some other by-products, and water. The separated complex is purified through washing. The com-

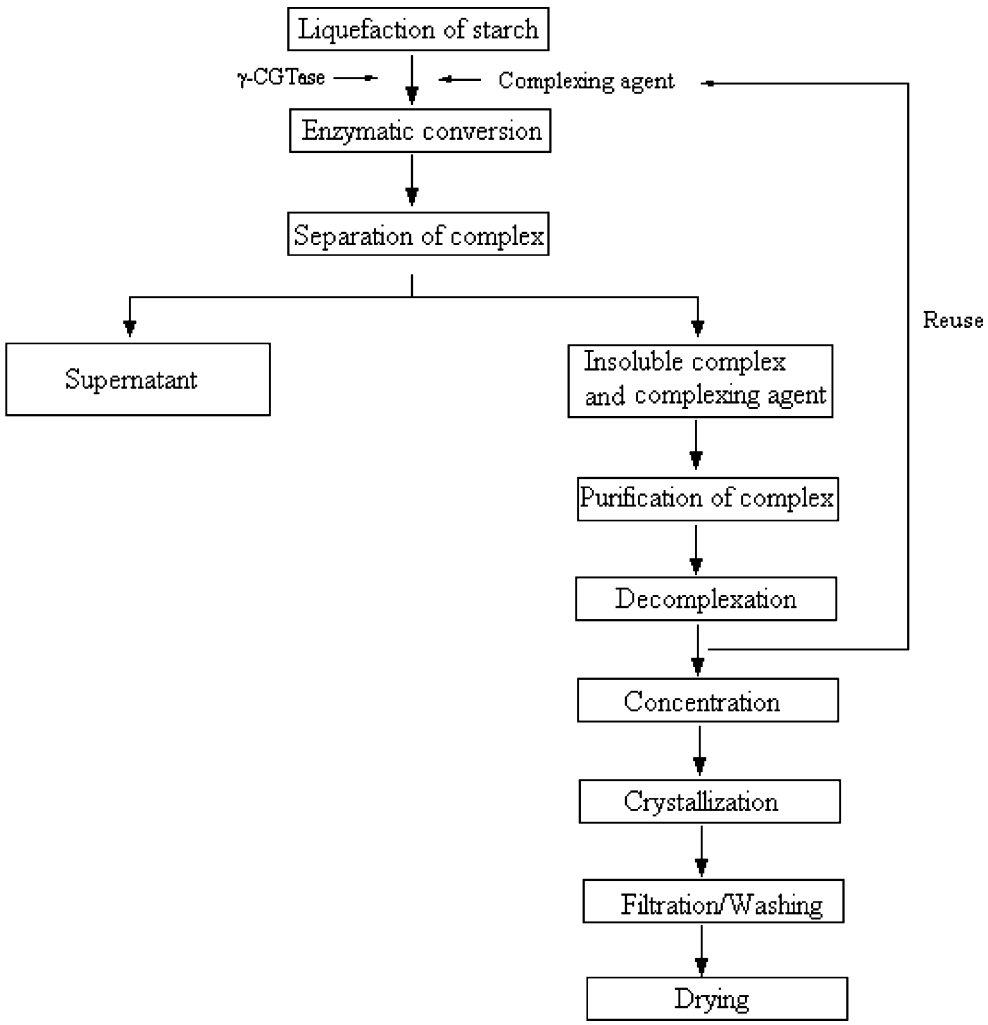

Fig. 3 The solvent process for γ-cyclodextrin production

plexing agent is then separated from γ-cyclodextrin by azeotropic distillation or liquid–liquid extraction with appropriate organic solvents such as acetone and *n*-decane. The remaining solution is concentrated via vacuum distillation, and γ-cyclodextrin is obtained as a white power by subsequent crystallization, filtration, washing, and drying.

Properties of γ-cyclodextrin

Compared with α- and β-cyclodextrins, γ-cyclodextrin has unique properties. First, γ-cyclodextrin possesses a huge advantage of having a larger internal cavity (Table 2), which can trap larger molecules that cannot be trapped by α- and β-cyclodextrins. Based on the dimensions of their cavities, α-cyclodextrin can form inclusion complexes only with low-molecular-weight molecules or compounds with aliphatic side chains, and β-cyclodextrin can complex aromatics or heterocycles, while γ-cyclodextrin can accommodate a wider variety of large organic compounds such as macrocycles and steroids (Martin Del Valle 2004). Second, γ-cyclodextrin has a noncoplanar and more flexible structure, which gives it the much higher solubility (232 g/l, 25°C) than α-cyclodextrin (145 g/l, 25°C) and β-cyclodextrin (18.5 g/l, 25°C; Szejtli 1982). The extra high solubility of γ-cyclodextrin facilitates it as the host to prepare highly concentrated solutions of active guest molecules (Szejtli 1998), which further promotes its applications in many industries. Third, γ-cyclodextrin can be rapidly and essentially completely digested by human salivary amylase and pancreatic amylase, which are unable to digest α-cyclodextrin and β-cyclodextrin to any measurable extent (Marshall and Miwa 1981; Kondo et al. 1990). Thus, γ-cyclodextrin is rapidly degraded and absorbed in the human small intestine, unlike α-cyclodextrin and β-cyclodextrin, which are generally recognized to be nondigestible (De Bie et al. 1998; Lai et al. 2005). The high bioavailability of γ-cyclodextrin makes it ideal for some specific applications in the food and pharmaceutical industries. In addition, γ-cyclodextrin does not represent a hazard to human health based on detailed and reassuring toxicity data (Waalkens-Berendsen et al. 1998a,b). It can be well tolerated up to 20% of the diet without any adverse effects (Munro et al. 2004).

Table 2 Three-dimensional form and size of cyclodextrins

Properties	α-Cyclodextrin	β-Cyclodextrin	γ-Cyclodextrin
Internal diameter (Å)	4.7–5.3	6.0–6.5	7.5–8.3
External diameter (Å)	14.6	15.4	17.5
Cavity Height (Å)	7.9	7.9	7.9
Approx. cavity volume ($Å^3$)	174	262	427

Values from Szejtli (1982).

Applications

Because γ-cyclodextrin, compared with α- and β-cyclodextrins, has a larger cavity, higher water solubility, and more bioavailability, it has wider applications in many industries, especially in the food and pharmaceutical industries.

Food

Because γ-cyclodextrin is declared to be "General Recognized As Safe" and has no adverse effects on the absorption of certain nutrients in a variety of food and nutraceutical applications (Munro et al. 2004), it has been proposed that it can be used in all kinds of food and nutraceutical applications as a food ingredient and additive (Food Standards in Australia New Zealand 2003).

γ-cyclodextrin can be used as a carrier and stabilizer for many bulky guests such as fat-soluble vitamins (e.g., vitamin D2, tocopherols, and tocotrienol), polyunsaturated fatty acids (e.g., omega-3, 6, and 9 fatty acids), sensitive colors (e.g., lycopene, lutein, and anthocyanin), and unique flavors from herbs, spices, fruits, etc. Although α- and β-cyclodextrins can be used to complex with vitamins and polyunsaturated fatty acids (PUFAs) triglycerides from algae, fish, or vegetable sources, only γ-cyclodextrin can achieve a nearly complete complexation and provide the best stabilization against autoxidation during storage (O'Donnell 2001). An additional benefit of the complexation of vitamins and PUFAs triglycerides with γ-cyclodextrin is the formation of stable dispersions of fish or vegetable oils in aqueous media (Regiert et al. 1996). Furthermore, when used in food formulations, γ-cyclodextrin can stabilize and protect certain sensitive colors and unique flavors throughout many rigorous food-processing procedures such as freezing, thawing, and microwaving, which allows the quality and quantity of color or flavor to be preserved to a greater extent and longer period compared to α- and β-cyclodextrins (Thoss et al. 1993; Muoz-Botella et al. 1995; Tamura et al. 1999).

γ-Cyclodextrin can also stabilize emulsions of fats and oils. This property is useful for the preparation of bread spreads (Munro et al. 2004). In ready-to-eat dairy desserts or in desserts prepared from dry mixes with the admixture of milk, γ-cyclodextrin stabilizes the fat/water emulsion and the foam at levels up to 3%. In frozen dairy desserts, γ-cyclodextrin improves the melting behavior at a concentra-

tion of less than 3%. In addition, γ-cyclodextrin can also improve the retention of water or fat in some filling foods. In fruit fillings, not more than 3% γ-cyclodextrin is required for achieving the intended effect. In fat fillings, up to 5% γ-cyclodextrin may be required for preventing the so-called oiling-out.

γ-Cyclodextrin can be rapidly digested and absorbed in the human small intestine. However, it is interesting to note that orally administered γ-cyclodextrin can provide a blunted postprandial glucose response and reduced insulin secretion, which is more similar to that of a slowly digested carbohydrate rather than a rapidly digested carbohydrate. Therefore, nutritional products can be formulated with γ-cyclodextrin to provide diabetics or other suitable individuals with a nutrition source that delivers a blunted postprandial glycemic response. These products allow for better control over blood glucose fluctuations, both hyper- and hypoglycemic swings, after eating a meal or snack. Thus, it is especially useful in individuals prone to such glycemic swings (Lai et al. 2005).

Pharmaceutical

The application of γ-cyclodextrins in the pharmaceutical industry has been intensively studied. As a result of its unique properties, γ-cyclodextrin is the best carrier for drugs compared to α- and β-cyclodextrins.

γ-Cyclodextrin can be used to significantly improve the solubility and dissolution of drugs. For example, C_{60} exhibits an interesting range of biological activities, especially promising in the field of anti-human immunodeficiency virus activity, photo dynamic therapy, etc. (Friedman et al. 2003); however, its low solubility in water hampers the research on its biological activities. γ-Cyclodextrin can form inclusion complex with the C_{60} molecule, which cannot fit into the cavity of α- or β-cyclodextrin, and this supermolecular complex is water soluble (Komatsu et al. 1999; Suvegh et al. 2001). Similar solubility enhancement has been also found with digoxin (Uekama et al. 1983), oxazepam (Moyano Mendez et al. 1995), praziquantel (Becket et al. 1999), and omeprazole (Arias et al. 2000). Thus, the formulation of poorly water-soluble drugs with γ-cyclodextrin can effectively improve their apparent solubility/dissolution and prevent crystallization of active ingredients.

In many cases, as a result of cyclodextrin-increased drug solubility and dissolution, γ-cyclodextrin can significantly enhance the bioavailability of poorly water-soluble drugs and improve their pharmacological effects, which allows a reduction in the dose of the drug. For example, coenzyme Q10, an endogenous component that plays an important part in mitochondrial electron transport, has been employed for treating heart disease and degenerative disorders (Rosenfeldt et al. 2003). Although the formulations with all three types of cyclodextrins are resistant to air/light-induced degradation and have a high water solubility, only γ-cyclodextrin can significantly improve the bioavailability of coenzyme Q10 (Terao et al. 2006). In fact, the bioavailability of coenzyme Q10 in complexation with γ-cyclodextrin is enhanced by almost 4.2-fold compared to that of the free substance (Moldenhauer and Cully 2003) and by about 35% compared to a microcrystalline cellulose–coenzyme Q10 complex (Terao et al. 2006). In addition, it has also been found that γ-cyclodextrin can improve bioavailability of drugs by enhancing their membrane permeability (Matsuda and Arima 1999; Challa et al. 2005).

γ-Cyclodextrin can improve the stability of active pharmaceutical ingredients and increase the shelf life of drugs (Loftsson and Brewester 1996). By providing a molecular shield, γ-cyclodextrin complexation encapsulates labile drug molecules at the molecular level and thus insulates them against various degradation processes (Challa et al. 2005). For example, γ-cyclodextrin can increase the resistance of digoxin (Uekama et al. 1983), spiranolactone (Jarho et al. 2000) and paclitaxel (Singla et al. 2002) to hydrolysis, oxidation, heat, light, and metal salts. Additionally, encapsulating oils/liquids or volatile active ingredients in γ-cyclodextrin molecules can convert them to solid powders that can be conveniently formulated into stable tablets (Matsuda and Arima 1999).

γ-Cyclodextrin is also used to ameliorate the irritation caused by drugs more effectively than α- and β-cyclodextrin (Rajewski and Stella 1996). On the one hand, the increased drug efficacy and potency, caused by γ-cyclodextrin-increased drug solubility, can reduce drug toxicity by lowering the drug doses; on the other hand, because active ingredients that irritate the stomach, skin, or eye are encapsulated within γ-cyclodextrin molecules, the local concentration of free active ingredient is decreased below the irritancy threshold. In addition, γ-cyclodextrin can be used to reduce the bitter or irritant taste and bad smell of drugs (Irie and Uekama 1999).

Other applications

In the cosmetic industry, the formation of an inclusion complex between γ-cyclodextrin and a broad variety of organic compounds increases the stability and solubility of cosmetic active ingredients and provides a better control over the release of fragrances (Buschmann and Schollmeyer 2002). For example, lipophilic vitamins such as retinol and tocopherol are essential in skin care products because of their nature as a free-radical scavenger; however, they are sensitive to light- and oxidation-induced degradation. The disadvantage prevents their effectiveness in cosmetic preparations. By forming inclusion complexes with cyclodextrins, these vitamins are effectively protected against

deterioration (Regiert and Moldenhauer 1998; Regiert and Kupka 2003, 2004; Regiert 2005). Compared with α- and β-cyclodextrins, γ-cyclodextrin can achieve better stabilization of lipophilic vitamins, which makes it possible for lipophilic vitamins to be widely used in cosmetics (Moldenhauer et al. 1998).

In the chemical industry, γ-cyclodextrins can be used in separation processes because of its ability to discriminate between positional isomers, functional groups, homologues, and enantiomers (Han 1997). It serves as an ideal selector by molecular recognition and further enhances the complex-forming ability and selectivity in various types of separations (Schneiderman and Stalcup 2000). Chiral separation is one of the most important areas of application for γ-cyclodextrin. γ-Cyclodextrin has been frequently used in high-performance liquid chromatography, capillary electrophoresis, and magnetic resonance imaging (MRI) for the separation of chiral compounds. For example, with γ-cyclodextrin as a chiral-solvating agent in capillary zone electrophoresis, a number of chiral drugs were enantioseparated successfully (Koppenhoefer et al. 1995, 1998). γ-Cyclodextrin was also used to perform chiral discrimination of (±)-5,6-diisobutyroyl-2-methylaminotetralin hydrochloride by H-1-MRI, and the 95% enantiomeric excess of the (−)-isomer was determined successfully (Redenti et al. 1992).

In the textile industry, γ-cyclodextrin is a new auxiliary substance. It can serve as a warp size for fabric formation and as a latent colorant for polyester. For example, George et al. (2004) found that γ-cyclodextrin could function simultaneously as a warp size and a latent colorant to combine the weaving and the ink jet-printing steps, whereas the usage of α- and β-cyclodextrin was limited because many dye molecules could not fit into their relatively small cavities. γ-Cyclodextrin can also be used to provide good textile finishing to cottons, woolens, and blended materials. When bound chemically to fibers, it can provide enhanced hydrophilicity and form inclusion complex to immobilize perfumes, insect repellents, antimicrobial agents, etc (Buschmann et al. 2001). In addition, γ-cyclodextrin can improve the cord strength of polyester fibers used for reinforcement of rubbers (Szejtli 2003).

γ-Cyclodextrin is also attractive for various applications in many other fields: agricultural industry, bioconversions and fermentations, environmental protection, adhesives, coatings, polymers, etc. In the agricultural industry, compared with α- and β-cyclodextrins, γ-cyclodextrin can form inclusion complexes with a wider variety of agricultural chemicals including herbicides and other plant-regulating agents, insecticides, and fungicities. Thus, it can reduce the contact hazards of these agricultural chemicals and afford a controlled release (McMahon et al. 1995). With respect to bioconversions and fermentations, γ-cyclodextrin can enhance solubilization of organic compounds and reduce toxicity by complexation with toxins (Bar 1989). Prabhu and Ramadoss (2000) found that there was distinct increase in the rate of formation of penicillin-G from phenylacetic acid and 6-aminopenicillanic acid if both substrates used are in a γ-cyclodextrin-complexed form. In environmental science, γ-cyclodextrin is used to enrich and remove organic pollutants and heavy metals from soil, water, and atmosphere. Fava et al. (1998) found that γ-cyclodextrin had the potential of being successfully used in the bioremediation of chronically polychlorinated biphenyl-contaminated soils. In adhesive and coating industries, γ-cyclodextrin can increase the tackiness and adhesion of some hot melt adhesives and control the release of some odors in some pressure-sensitive adhesives (Lipman 2000). It is also suitable for removal of the particular odor problems, which arise with coating materials such as stains, sealants, nail polish, and, especially, paints (Uchiyama et al. 2002). In addition, the formation of γ-cyclodextrin–polymer inclusion complexes represents a novel way to manipulate the properties of several high-molecular-weight polymers such as polypropylene, poly(butene-1), and polyethylene. These polymers inclusion into γ-cyclodextrin can extend and reorganize their conformations and, thus, improve their commercial properties (Rusa et al. 2004).

Prospective

The capability of forming complexes with a wider variety of organic compounds, together with relatively high water solubility and more bioavailability, have resulted in rapidly increasing application potential of γ-cyclodextrin in many fields, especially in the food and pharmaceutical industries. However, its application is still significantly limited because of its low yield and extravagant price. It is expected that advancements in biotechnology will dramatically improve the manufacturing process of highly pure γ-cyclodextrin and expand its industrial applications.

Acknowledgments We thank Dr Y. Zhu (Wageningen University, The Netherlands) for critically reading this manuscript. This work was supported financially by the Natural Science Foundation of Jiangsu Province (BK2007019), the Major State Basic Research Development Program of China (973 Program; 2007CB714306), and the National High-tech Research and Development Program of China (863 Program; 20060110Z3008).

Reference

Arias MJ, Moyano JR, Munoz P, Gines JM, Justo A, Giordano F (2000) Study of omeprazole–gamma-cyclodextrin complexation in the solid state. Drug Dev Ind Pharm 26:253–259

Bar R (1989) Cyclodextrin aided bioconversions and fermentations. Trends Biotechnol 7:2–4

Becket G, Schep LJ, Tan MY (1999) Improvement of the in vitro dissolution of praziquantal by complexation with alpha-, beta and gamma-cyclodextrins. Int J Pharm 179:65–71

Bender H (1983) An improved method for the preparation of cyclooctaamylose, using starches and the cyclodextrin glycosyltransferase of *Klebsiella pneumoniae* M 5 al. Carbohydr Res 124:225–233

Biwer A, Antranikian G, Heinzle E (2002) Enzymatic production of cyclodextrins. Appl Microbiol Biotechnol 59:609–617

Buschmann HJ, Schollmeyer E (2002) Applications of cyclodextrins in cosmetic products: a review. J Cosmet Sci 53:575–592

Buschmann HJ, Knittel D, Schollmeyer E (2001) New textile applications of cyclodextrins. J Incl Phenom Macrocycl Chem 40:169–172

Cao XZ, Jin ZY, Wang X, Chen F (2005) A novel cyclodextrin glycosyltransferase from an alkalophilic *Bacillus species*: purification and characterization. Food Res Int 38:309–314

Challa R, Ahuja A, Ali J, Khar RK (2005) Cyclodextrins in drug delivery: an updated review. AAPS PharmSciTech 6:E329–E357

De Bie AT, van Ommen B, Bär A (1998) Disposition of ^{14}C-gamma-cyclodextrin in germ-free and conventional rats. Regul Toxicol Pharmacol 27:150–158

Endo T, Zheng M, Zimmermann W (2002) Enzymatic synthesis and analysis of large-ring cyclodextrins. Aust J Chem 55:39–48

Englbrecht A, Harrer G, Lebert M (1988) Biochemical and genetic characterization of a CGTase from an alkalophilic bacterium forming primarily γ-cyclodextrin. In: Huber O, Szejtli J (eds) Proceedings of the fourth international symposium on cyclodextrins. Kluwer, Dordrecht, pp 87–92

Fava F, Gioia D, Marchetti L (1998) Cyclodextrin effects on the ex-situ bioremediation of a chronically polychlorobiphenyl-contaminated soil. Biotechnol Bioeng 58:345–55

Food Standards in Australia New Zealand (2003) Final assessment report, application a438: gamma cyclodextrin as a novel food ingredient/food additive. March 19

French D (1957) The Schardinger dextrins. Adv Carbohydr Chem 12:189–260

Friedman SH, Schinazi RF, Wudl F, Hill CL, DeCamp DL, Sijbesma RP, Kenyon GL (2003) Method of treatment of viral infection including hiv using water soluble fullerenes. US Patent 6613771

Fugita Y, Tsubouchi H, Inagi Y, Tomita K, Ozaki A, Nakanishi K (1990) Purification and properties of cyclodextrin glycosyltransferase from *Bacillus sp.* AL-6. J Ferment Bioeng 70:150–154

George B, Govindaraj M, Ujiie H, Freeman H, Hudson S (2004) Integration of fabric formation and coloration processes. NTC Research Briefs June:C02-PH03

Goel A, Nene S (1995) A novel cyclomaltodextrin glucanotransferase from *Bacillus firmus* that degrades raw starch. Biotechnol Lett 17:411–416

Han SM (1997) Direct enantiomeric separations by high performance liquid chromatography using cyclodextrins. Biomed Chromatogr 11:259–271

Harata K, Haga K, Nakamura A, Aoyagi M, Yamane M (1996) X-Ray structure of cyclodextrin glucanotransferase from alkalophilic *Bacillus sp.* 1011. Comparison of two independent molecules at 1.8 Å resolution. Acta Crystallogr D Biol Crystallogr 52:1136–1145

Hedges AR (1998) Industrial applications of cyclodextrins. Chem Rev 98:2035–2044

Hirano K, Ishihara T, Ogasawara S, Maeda H, Abe K, Nakajima T, Yamagata Y (2005) Molecular cloning and characterization of a novel γ-CGTase from alkalophilic *Bacillus sp*. Appl Microbiol Biotechnol 67:1–9

Irie T, Uekama K (1999) Cyclodextrins in peptide and protein delivery. Adv Drug Deliv Rev 36:101–123

Jarho P, Vander Velde D, Stella VJ (2000) Cyclodextrin-catalyzed deacetylation of spironolactone is pH and cyclodextrin dependent. J Pharm Sci 89:241–249

Kato T, Horikoshi K (1986) A new γ-cyclodextrin forming enzyme prodiced by *Bacillus subtilis* no. 313. . J Jpn Soc Starch Sci 34:137–143

Kim MH, Sohn CB, Oh TK (1998) Cloning and sequencing of a cyclodextrin glycosyltransferase gene from *Brevibacillus brevis* CD162 and its expression in *Escherichia coli*. FEMS Microbiol Lett 164:411–418

Komatsu K, Fujiwara K, Murata Y, Braun T (1999) Aqueous solubilization of crystalline fullerenes by supramolecular complexation with γ-cyclodextrin and sulfocalix under mechanochemical high speed vibration milling. J Chem Soc Perkin Trans 1:2963–2966

Kondo H, Nakatani H, Hiromi K (1990) In vitro action of human and porcine α-amylases on cyclo-maltooligosaccharides. Carbohydr Res 204:207–213

Koppenhoefer B, Epperlein U, Christian B, Ji Y, Chen Y, Lin B (1995) Separation of enantiomers of drugs by capillary electrophoresis: part I. Gamma-cyclodextrin as chiral solvating agent. J Chromatogr 717:181–190

Koppenhoefer B, Epperlein U, Jakob A, Wuerthner S, Zhu X, Lin B (1998) Separation of enantiomers of drugs by capillary electrophoresis, part 7: gamma-cyclodextrin as chiral solvating agent. Chirality 10:548–554

Lawson CL, van Montfort R, Strokopytov B, Rozeboom HJ, Kalk KH, de Vries GE, Penninga D, Dijkhuizen L, Dijkstra BW (1994) Nucleotide sequence and X-ray structure of cyclodextrin glycosyltransferase from *Bacillus circulans* strain 251 in a maltose-dependent crystal form. J Mol Biol 236:590–600

Lai CS, Chow JM, Wolf BW (2005) Method of using gamma cyclodextrin to control blood glucose and insulin secretion. US Patent 20050215523 A1

Lipman RD (2000) Cyclodextrin containing pressure sensitive adhesives. Eur Patent EP1206290

Loftsson T, Brewester M (1996) Pharmaceutical applications of cyclodextrins. 1. Drug solubilization and stabilization. J Pharm Sci 85:1017–1025

Marshall JJ, Miwa I (1981) Kinetic difference between hydrolyses of γ-cyclodextrin by human salivary and pancreatic α-amylases. Biochim Biophys Acta 661:142–147

Martins RF, Hatii-Kaul R (2002) A new cyclodextrin glycosyltransferase from an alkaliphilic *Bacillus agaradhaerens* isolate: purification and characterization. Enzyme Microb Technol 30:116–124

Martin Del Valle EM (2004) Cyclodextrins and their uses: a review. Process Biochem 39:1033–1046

Matioli G, Zanin GM, Moraes FF (2000) Enhancement of selectiveity for producing γ-cyclodextrin. Appl Biochem Biotech 84–86: 955–962

Matsuda H, Arima H (1999) Cyclodextrins in transdermal and rectaldelivery. Adv Drug Deliv Rev 36:81–99

McMahon WA, Lew CW, Branly KL (1995) Controlled release microcapsules. US Patent 5466460

Moldenhauer JP, Cully J (2003) Method for producing a coenzyme Q10/γ-cyclodextrin complex. US Patent 20030012774 A1

Moldenhauer JP, Regiert M, Wimmer T (1998) Complexes of gamma-cyclodextrin and retinol or retinol derivatives, processes for their preparation and their use. US Patent 5985296

Mori S, Hirose S, Oya T, Kitahata S (1994) Purification and properties of cyclodextrin glucanotransferase from *Brevibacterium sp*. No. 9605. Biosci Biotech Biochem 58:1968–1972

Moyano Mendez JR, Arias Blanco MJ, Gines Dorado JM, Rabasco Alvarez AM (1995) Application of γ-cyclodextrin to the improvement of dissolution characteristics of oxazepam. Il Farmaco 50:791–799

Munro IC, Newberne PM, Young VR, Bär A (2004) Safety assessment of γ-cyclodextrin. Regul Toxicol Pharm 39:S3–S13

Muoz-Botella S, del Castillo B, Martyn MA (1995) Cyclodetrin properties and applications of inclusion complex formation. Ars Pharm 36:187–198

Nakagawa Y, Takada M, Ogawa K, Hatada Y, Horikoshi K (2006) Site-directed mutations in Alanine 223 and Glycine 255 in the acceptor site of gamma-cyclodextrin glucanotransferase from Alkalophilic *Bacillus clarkii* 7364 affect cyclodextrin production. J Biochem (Tokyo) 140:329–336

O'Donnell CD (2001) New encapsulating molecule improves taste. prepared foods july from website: http://findarticles.com/p/articles/mi_m3289/is_7_170/ai_77107380

Parsiegla G, Schmidt AK, Schulz GE (1998) Substrate binding to a cyclodextrin glycosyltransferase and mutations increasing the γ-cyclodextrin production. Eur J Biochem 255:710–717

Penninga D, Strokopytov B, Rozeboom HJ, Lawson CL, Dijkstra BW, Bergsma J, Dijkhuizen L (1995) Site directed mutations in tyrosine 195 of cyclodextrin glycosyltransferase from *Bacillus circulans* strain 251 affect activity and product specificity. Biochemistry 34:3368–3376

Prabhu KS, Ramadoss CS (2000) Penicillin acylase catalyzed synthesis of penicillin-G from substrates anchored in cyclodextrins. Indian J Biochem Biophys 37:6–12

Qi Q, Zimmermann W (2005) Cyclodextrin glucanotransferase: from gene to applications. Appl Microbiol Biotechnol 66:475–485

Qi Q, Mokhtar MN, Zimmermann W (2007) Effect of ethanol on the synthesis of large-ring cyclodextrins by cyclodextrin glucanotransferases. J Incl Phenom Macrocycl Chem 57:95–99

Rajewski RA, Stella VJ (1996) Pharmaceutical applications of cyclodextrins.2. In vivo drug delivery. J Pharm Sci 85:1142–68

Redenti E, Fronza G, Bovis G, Ventura P (1992) Application of γ-cyclodextrin to enantiomeric purity determination of a new 2-amino-tetralin derivative by H-1-NMR spectroscopy. Chirality 4:404–405

Regiert M (2005) Light-stable vitamin E by inclusion in gamma-cyclodextrin. SOEFW Int J Appl Sci 131:10–18

Regiert M, Kupka M (2003) 2:1-Inclusion compound from beta- or gamma-cyclodextrin and alpha-tocopherol. US Patent 20030130231A1

Regiert M, Kupka M (2004) Cosmetic composition comprising a complex of cyclodextrin and vitamin F. US Patent 20040096413 A1

Regiert M, Moldenhauer JP (1998) Inclusion compound from gamma-cyclodextrin and retinol, production and application. Eur Patent EP0867175 A1, DE19847633 C1

Regiert M, Wimmer T, Moldenhauer JP (1996) Application of γ-cyclodextrin for the stabilization and/or dispersion of vegetable oils containing triglycerides of polyunsaturated acids. J Inclu Pheno Mol Recogn Chem 25:213–216

Rendleman JA Jr (1992) Enhanced production of cyclomaltooctaose (γ-cyclodextrin) through selective complexation with C_{12} cyclic compounds. Carbohyd Res 230:343–359

Rendleman JA Jr (1993) Enhanced production of γ-cyclodextrin from corn syrup solids by means of cyclododecanone as selective complexant. Carbohydr Res 247:223–237

Rosenfeldt F, Hilton D, Pepe S, Krum H (2003) Systematic review of effect of coenzyme Q10 in physical exercise, hypertension and heart failure. Biofactors 18:91–100

Rusa CC, Rusa M, Gomez M, Shin I, Fox J, Tonelli AE (2004) Nanostructuring high molecular weight isotactic polyolefins via processing with γ-cyclodextrin inclusion compounds. formation and characterization of polyolefin– γ-cyclodextrin inclusion compounds. Macromolecules 37:7992–7999

Sato M, Yagi Y (1991) Properties of CGTase from three types of Bacillus and production of cyclodextrins by the enzymes. In: Friedman RB (ed) Biotechnology of amylodextrin oligosaccharides, ACS symposium series 458. American Chemical Society, Washington, DC, pp 125–137

Schmid G (1996) Preparation and industrial production of cyclodextrins. In: Atwood J, Davies ED, MacNicol DD, Vogtle F (eds) Comprehesive supermolecular chemistry, vol 3: Cyclodextrins. Pergamon, Oxford, pp 41–56

Schneiderman E, Stalcup AM (2000) Cyclodextrins: a versatile tool in separation science. J Chromatogr B 745:83–102

Shieh W (1996) Process for producing gamma-cyclodextrin. US Patent 5550222

Singla AK, Garg A, Aggarwal D (2002) Paclitaxel and its formulations. Int J Pharm 235:179–192

Strokopytov B, Knegtel RM, Penninga D, Rozeboom HJ, Kalk KH, Dijkhuizen L, Dijkstra BW (1996) Structure of cyclodextrin glycosyltransferase complexed with a maltononaose inhibitor at 2.6 angstrom resolution. Implications for product specificity. Biochemistry 35:4241–4249

Suvegh K, Fujiwara K, Komatsu K, Marek T, Ueda T, Vertes A, Braun T (2001) Positron lifetime in supramolecular gamma- and delta-cyclodextrin-C_{60} and C_{70} compounds. Chem Phys Lett 344: 263–269

Szejtli J (1982) Cyclodextrins and their inclusion complexes. Akademiai Kiado, Budapest

Szejtli J (1998) Introduction and general overview of cyclodextrin chemistry. Chem Rev 98:1743–1753

Szejtli J (2003) Cyclodextrins in the textile industry. Starch/Stärke 55:191–196

Szejtli J (2004) Past, present, and future of cyclodextrin research. Pure Appl Chem 76:1825–1845

Szente L, Szejtli J (2004) Cyclodextrins as food ingredients. Trends Food Sci Tech 15:137–142

Takada M, Ide T, Yamamoto T, Unno T, Watanabe Y, Sone H, Yamamoto M (2003a) Novel cyclodextrin glucanotransferase, process for producing the same and process for producing cyclodextrin by using this enzyme. US Patent 20030194796 A1

Takada M, Nakagawa Y, Yamamoto T (2003b) Biochemical and genetic analyses of a novel γ-cyclodextrin glucanotransferase from an alkalophilic *Bacillus clarkii* 7364. J Biochem (Tokyo) 133:317–324

Tamura H, Takada M, Shiomi K (1999) The color stability and antioxidative activity of an anthocyanin and γ-cyclodextrin complex. In: Shibamoto T, Terao J, Osawa T (eds) Functional foods for disease prevention 1: fruits, vegetables, and teas, ACS symposium series 701. American Chemical Society, Washington, DC, pp 157–171

Terada Y, Yanase M, Takata H, Takaha T, Okada S (1997) Cyclodextrins are not the major cyclic α-1,4-glucans produced by the initial action of cyclodextrin glucanotransferase on amylose. J Biol Chem 272:15729–15733

Terao K, Nakata D, Fukumi H, Schmid G, Arima H, Hirayama F, Uekama K (2006) Enhancement of oral bioavailability of coenzyme Q_{10} by complexation with γ-cyclodextrin in healthy adults. Nutr Res 26:503–508

Thoss M, Schwabe L, Fromming KH (1993) Cyclodextrin-Einschlussverbindungen von Zitronen-, Orangen-, Hopfen- und Kamillenol. Pharm Ztg Wiss 6:144–148

Tonkova A (1998) Bacterial cyclodextrin glucanotransferase. Enzyme Micro Technol 22:678–686

Uchiyama H, Jensen JM, DuVal DL, Cetti JR, Woo RA, Archambault DL (2002) Reduction of odors from coating material. US Patent 20020132861 A1

Uekama K, Fujinaga T, Hirayama F, Otagiri M, Yamasaki M, Seo H, Hashimoto T, Tsuruoka M (1983) Improvement of the oral bioavailability of digitalis glycosides by cyclodextrin complexation. J Pharm Sci 72:1338–1341

Uitdehaag JC, Mosi R, Kalk KH, van der Veen BA, Dijkhuizen L, Withers SG, Dijkstra BW (1999a) X-Ray structures along the reaction pathway of cyclodextrin glycosyltransferase elucidate catalysis in the α-amylase family. Nat Struct Biol 6:432–436

Uitdehaag JC, Kalk KH, van Der Veen BA, Dijkhuizen L, Dijkstra BW (1999b) The cyclization mechanism of cyclodextrin glycosyltransferase (CGTase) as revealed by a γ-cyclodextrin-CGTase complex at 1.8-A resolution. J Biol Chem 274:34868–34876

Uitdehaag JC, van Alebeek GJ, van Der Veen BA, Dijkhuizen L, Dijkstra BW (2000a) Structures of maltohexaose and maltoheptaose bound at the donor sites of cyclodextrin glycosyltransferase give insight into the mechanisms of transglycosylation activity and cyclodextrin size specificity. Biochemistry 39:7772–7780

Uitdehaag JC, van der Veen BA, Dijkhuizen L, Dijkstra BW (2000b) Catalytic mechanism and product specificity of cyclodextrin glycosyltransferase, a prototypical transglycosylase from the α-amylase family. Enzyme Microb Technol 30:295–304

van der Veen BA, Uitdehaag JC, Dijkstra BW, Dijkhuizen L (2000a) Engineering of cyclodextrin glycosyltransferase reaction and product specificity. Biochim Biophys Acta 1543:336–360

van der Veen BA, van Alebeek GJ, Uitdehaag JC, Dijkstra BW, Dijkhuizen L (2000b) The three transglycosylation reactions catalyzed by cyclodextrin glycosyltransferase from *Bacillus circulans* (strain 251) proceed via different kinetic mechanisms. Eur J Biochem 267:658–665

van der Veen BA, Uitdehaag JC, Dijkstra BW, Dijkhuizen L (2000c) The role of arginine 47 in the cyclization and coupling reactions of cyclodextrin glycosyltransferase from *Bacillus circulans* strain 251. Implications for product inhibition and product specificity. Eur J Biochem 267:3432–3441

Waalkens-Berendsen DH, Smits-van Prooije AE, Bär A (1998a) Embryotoxicity and teratogenicity study with γ-cyclodextrin in rabbits. Regul Toxicol Pharmacol 27:172–177

Waalkens-Berendsen DH, Verhagen FJ, Bär A (1998b) Embryotoxicity and teratogenicity study with γ-cyclodextrin in rats. Regul Toxicol Pharmacol 27:166–171

Wang F, Du GC, Li Y, Chen J (2004) Optimization of cultivation conditions for the production of γ-Cyclodextrin Glucanotranferase by *Bacillus macorous*. Food Biotechnol 18:251–264

Wang F, Du GC, Li Y, Chen J (2005) Effects of dissolved oxygen tension and two-stage oxygen supply strategy on the production of γ-CGTase by *Bacillus macorous*. Process Biochem 40: 3468–3473

Wang F, Du GC, Li Y, Chen J (2006a) Regulation of CCR in the γ-CGTase production from *Bacillus macorous* by the specific cell growth rate control. Enzyme Microb Technol 39:1279–1285

Wang F, Du GC, Li Y, Chen J (2006b) Enhanced γ-CGTase production by *Bacillus Macorous* with membrane active substances. Food Biotechnol 20:171–181

Yang GW, Li J, Xie WM, Wang DM, Xie BT (2001) Studys on γ-CGTase from *Bacillus sp.* 32-3-1. Ind microbiol 31:30–32 (In Chinese)

Zheng M, Endo T, Zimmermann W (2002) Synthesis of large-ring cyclodextrins by cyclodextrin glucanotransferases from bacterial isolates. J Incl Phenom Macrocycl Chem 44:387–390