Carbohydrate Polymers **12** (1990) 375–392

The Cyclodextrins and their Applications in Biotechnology

J. Szejtli

Cyclolab, Cyclodextrin Research and Development Laboratory, 1026, Budapest, Endrõdi S. 38/40, Hungary

(Received 15 March 1989; accepted 13 June 1989)

ABSTRACT

Cyclodextrins and their derivatives enhance the solubility of complexed substrates in aqueous media, but do not damage the microbial cells or the enzymes. Therefore the enzymatic conversion of lipophilic substrates can be intensified (accelerated, or performed at higher substrate concentrations). Examples are the hydrolysis of triglycerides and lanatoside glycosides or the conversion of hydrocortisone to prednisolon and of cholesterol to androstenedione. In the presence of an appropriate cyclodextrin-derivative (e.g. 2,6-dimethyl-β-cyclodextrin). The lipid-like inhibitor substances are complexed, therefore the propagation of Bordatella pertussis *and the production of pertussis toxin increases up to 100-fold. Cyclodextrins or their fatty acid complexes can substitute mammalian serum in tissue cultures. A highly soluble γ-cyclodextrin–nystatin complex can protect tissue cultures from fungal infections. The tolerance level to toxic compounds during biological detoxication of organic chemical industries sewage can be elevated by admixing small amounts of β-cyclodextrin to the system, because the complexed toxic substances do not kill the detoxicating microbes.*

INTRODUCTION

The history of cyclodextrins is rather long (Szejtli, 1982; Bender & Komiyama, 1978) although they only became industrially available during the last decade. Their application in biotechnology only began in the 1980s but a rapid development is expected in this field (Szejtli, 1986, 1988).

The majority of biotechnology processes involve an enzyme-catalysed transformation of a substrate in aqueous medium. The main difficulties to arise are as follows:

Carbohydrate Polymers 0144-8617/90/$03.50

— the substrate is hydrophobic; sparingly (or practically not) soluble in water
— the enzyme or the enzyme producing microbial cells are sensitive to the toxic effects of the substrate or to inhibitors which may be the products of the transformation
— the substrate or the product is unstable under the enzymic transformation conditions.

Cyclodextrins are able to ensure a substrate solubilizing, and a substrate (or product) as well as microbial cell protecting effect in aqueous systems.

Cyclodextrins and their relevant properties

Cyclodextrins (CDs) are cyclic, nonreducing oligosaccharides. Three different CDs are known: α-, β- and γ-CD. All of them are produced industrially as homogeneous (purity over 99·5%) crystalline substances (Szejtli, 1982; Duchéne, 1987). See Fig. 1.

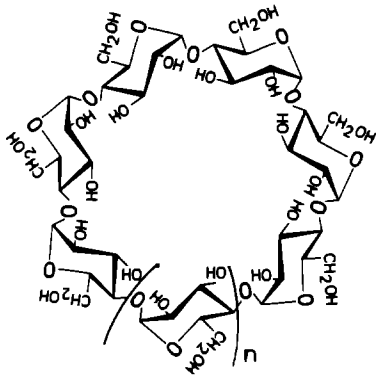

Fig. 1. Structure of CDs. n(no. of glycopyranosidic units) = 0 = α-CD; n = 1 = β-CD; n = 2 = γ-CD.

The α-CD consists of 6 (cyclomaltohexaose), the β-CD 7 (cyclomaltoheptaose) and the γ-CD 8 (cyclomaltoactaose) glucopyranose units. All secondary OH groups are located on one edge of the torus-like CD molecule, while all primary OH groups are on the other side. The 'lining' of the internal cavity is formed by H atoms, and glucosidic oxygen-bridge atoms, therefore this surface is slightly apolar (Fig. 2).

A CD molecule can be considered as an empty capsule of molecular size. When it is filled with the molecule of another substance it is called an 'inclusion complex'. Inclusion complexes are entities comprising two or more molecules, in which one of the molecules, the 'host' includes,

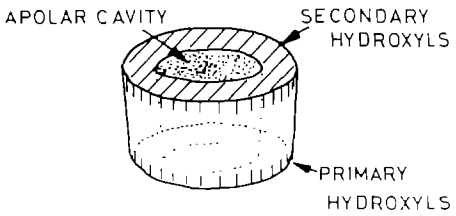

Fig. 2. Functional schematic representation of a CD 'capsule'.

totally or in part a 'guest' molecule, held only by physical forces, i.e. without covalent bonding. Cyclodextrins are typical 'host' molecules and may include a great variety of molecules having the size of a benzene ring, or a naphtalene molecule, or even larger ones which have a side chain of comparable size, to form crystalline inclusion complexes.

In aqueous solution the slightly apolar CD cavity is occupied by water molecules which are energetically unstable (polar–apolar interaction) and can therefore be readily substituted by appropriate 'guest molecules' which are less polar than water molecules. The dissolved CD is the 'host' molecule, and the 'driving force' of the complex formation is the substitution of the high-enthalpy water molecules by an appropriate 'guest' molecule (Fig. 3). The essence of the 'molecular encapsulation' is that one, two or three CD molecules contain one or more entrapped 'guest' molecules.

In aqueous CD solutions the solubility of lipophilic substances increases. The extent of this solubility enhancement depends on the type and concentration of the CD, temperature, etc. but mainly on the chemical structure of the substrate. Some examples are given in Table 1.

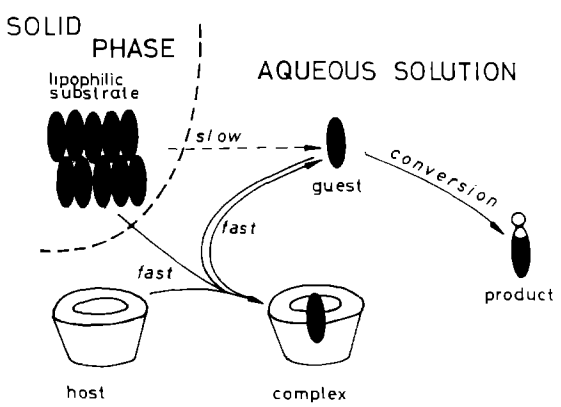

Fig. 3. Illustration of the host–guest interaction. The inclusion complex is a highly dynamic depot maintaining a constant concentration of the slowly dissolved 'free' guest.

TABLE 1

Solubility Enhancement of Some Drugs in 10% Aqueous Solutions of CD Derivatives (expressed as X-fold solubility enhancement versus uncomplexed, free drug)[a]

	Ibuprofen	*Tolnaftate*	*Indomethacin*	*Griseofulvin*	*Hydrocortisone*	*Dipiridamole*
β-CD	2·1	70	3·0	2·0	18	7·0
DIMEB	28	4600	22·5	4·6	87	218
TRIMEB	1·9	95	1·5	3·2	17	25
RAMEB	28	2600	16·0	4·0	43	87
SUMEB	27	2100	20·0	2·0	35	146
HPBCD-3,2	23	140	17·0	3·1	67	12
CDPS	17	400	—[b]	—	—	—
CDPSI-3,2	15	180	—	—	—	—

[a]In the case of β-CD, a saturated solution (~ 1.8% at 25°C).

[b]Not determined.

DIMEB, heptakis (2,6-di-*O*-methyl) β-CD; TRIMEB, heptakis (2,3,6-tri-*O*-methyl) β-CD; RAMEB; randomly methylated β-CD; SUMEB, monosuccinyl-DIMEB; HPBCD, hydroxypropyl-β-CD, *3*,2-hydroxypropyl-group per CD-ring; CDPS, epichlorohydrin-cross-linked, soluble β-CD-polymer, $\bar{M}_w \approx 5000$; CDPSI, carboxymethyl group containing CDPS, 3.2 or 5.2 carboxymethyl group per CD-ring.

Cyclodextrins generally do not interfere with the microbial cells at low concentrations, which can be taken into consideration for industrial purposes. There are however exceptions, when the CDs modify the structure and function of cell membranes, or strongly influence the metabolic activity of the cells. In most cases however the CDs can be considered to be inert toward the microbial cells in the aqueous substrate–enzyme systems. Cyclodextrins and particularly chemically modified CDs can damage e.g. the lipoprotein membrane of human erythrocytes at concentrations which strongly depend on the lipophilicity of the molecule. Table 2 illustrates the haemolytic activity of various CDs. As it is seen in Table 2, there are CDs and CD derivatives, which even at rather high concentrations are inert toward the erythrocyte membrane.

TABLE 2

Haemolytic Effects of CDs (0·4 ml suspension of human erythrocytes + 4 ml CD solution in 10 mM isotonic phosphate buffer, pH 7·4, 37°C, 30 min)

Cyclodextrin	*Cyclodextrin concentration (mg/ml)*	
	No haemolysis	*50% Haemolysis*
α-CD[a]	5·8	11·7
β-CD	1·8	7·8
γ-CD	11·0	32·0
DIMEB	0·3	1·44
SUMEB	1·0	6·5
TRIMEB	2·0	5·7
HPBCD-3,2	9·0	75
CDPS	7·0	37
CDPSI-3,2	12·0	75
CDPSI-5,2	15·0	150

[a]See footnote to Table 1.

The association–dissociation process in an aqueous substrate–CD system is a very dynamic one. The oscillation of a substrate molecule between its free and complexed state is very fast, therefore the complexation does not retard the enzymic transformation of the substrate. Notwithstanding the actual concentration of the 'free' substrate molecules (which is of crucial importance in determining the effect of the substrate on the cell membrane) its rate of uptake, or its toxicity is strongly reduced in the presence of CDs. Therefore the tolerable level of toxic substances or growth inhibitors (if they are complexable by CDs) can be

considerably elevated by adding CDs to the medium of the microbial cells. It means that the productivity of fermentation processes can be improved, or intolerable levels of toxic substances can be tolerated by the microbial cells in the presence of the appropriate type and concentration of CDs.

Cyclodextrins are more resistant to acidic or alkaline degradation than starch. Elevated temperatures (sterilization) up to the temperature of caramelization (> 200°C), in dry state, or in solution (between pH 2 and 12) results in no damage (Szejtli, 1982, 1988).

Only specific cyclodextrinase enzymes can rapidly degrade the CDs. Therefore in any biotechnological (microbial conversion, fermentation) process it should be ascertained whether the formation of such enzymes can be expected. Generally however CDs, and particularly the substituted CDs remain intact in most biotechnological processes which use homogeneous microbial populations, and therefore can eventually be recycled.

While the natural CDs show no surface activity, the alkylated CDs even at very low concentrations show marked surface tension (Uekama *et al.,* 1981; Szejtli, 1988). A methylated CD molecule itself is a micelle, a cylinder-like structure with its apolar cavity. Therefore methylated CDs in several aspects behave as surface active agents, but do not show any critical micelle concentration.

In the following, several examples will be presented for the application of CDs in enzymic or microbiological transformations of various substrates, where the aims are different: to enhance the yield, to accelerate the conversion, to protect the microorganisms or to substitute more expensive components of the medium.

MICROBIOLOGICAL SUBSTRATE CONVERSION

Figure 4 illustrates the solubility of hydrocortisone in various CD solutions. The dimethyl- β-CD (=heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin; commercial name: DIMEB) is too expensive (yet) for industrial purposes, but a very significant improvement of the microbiological conversion of hydrocortisone to prednisolon is based on the seemingly small solubility increase in β-CD solution.

The solubility of hydrocortisone in water is only 0·4 mg/ml, therefore in large volumes only a relatively small amount of this steroid could be converted. The conversion process was slow, and the end product was not homogeneous because of mixed-crystal formation (hydrocortisone–

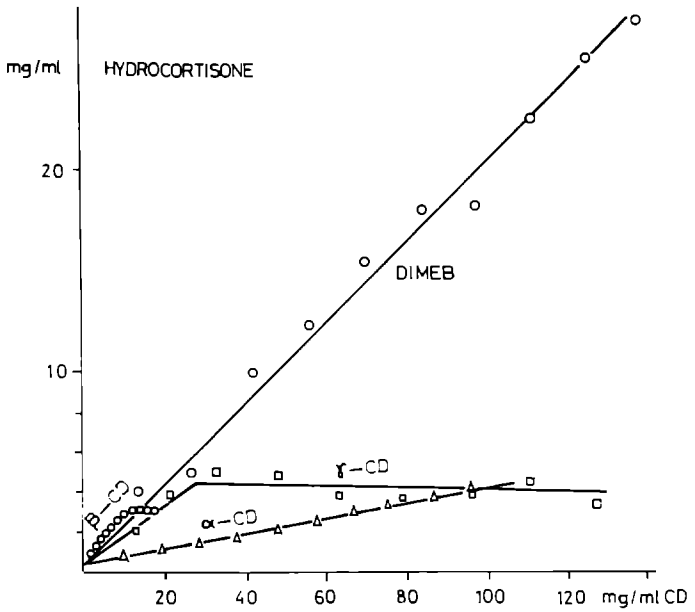

Fig. 4. Solubility of hydrocortisone in various CD-solutions, at room temperature after shaking with an excess of the steroid with CD-solution for 24 h.

prednisolon). Performing this process in an aqueous β-CD solution, the capacity is increased by more than 300%. The solubility of hydrocortisone increases, 3–4 times more hydrocortisone can be charged into the converter, the reaction is faster, and the end product is more homogeneous. Practically all microbiological steroid conversion processes have been tested in aqueous CD solutions with unanimously promising results (Udvardy *et al.*, 1983). The mentioned example is already practised in the pharmaceutical industry.

Recently the microbial conversion of cholesterol to androst-4-ene-3,17-dione has been studied in β-CD solution (Hesselink *et al.*, 1987). In the absence of CD, the bioconversion was subject to product inhibition and the steroid nucleus was degraded. Therefore only 40% of the cholesterol was converted to product in 180 h (Fig. 5). The presence of β-CD led to increased steroid solubilities and a more rapid bioconversion. A 95% conversion of cholesterol to product occurred.

A similar stimulatory effect of CDs was observed in a biotransformation of aromatic aldehydes to aromatic alcohols by growing yeast cells. The yeast fermentative activity and bioconversion were both faster in the presence of CDs. In both cases, more substrate was converted to product in the presence of CDs than without it (Bar, 1989).

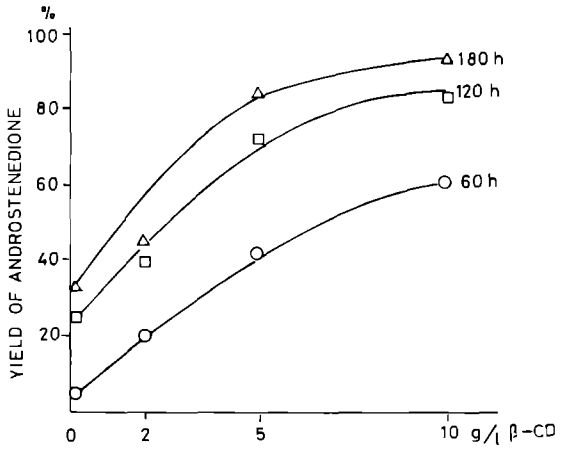

Fig. 5. Effect of β-CD-concentration on the conversion of cholesterol to androst-4-ene-3,17-dione by *Mycobacterium* sp. NRRL-B 3683, after 60, 120 and 180 h conversion time (Hesselink *et al.*, 1987).

FERMENTATION

The fatty acid synthesis of *Mycobacterium phlei (M. smegmatis)* is stimulated in the presence of CDs (Machida *et al.*, 1973) and even more so by alkylated CDs (Bergeron *et al.*, 1975).

The production of lankacidin-group antibiotics was markedly stimulated by adding β-CD to the fermentation medium (Sawada *et al.*, 1987). The production of lankacidin A and C by *Streptomyces rochei var. volubilis* was enhanced by adding 11 mM β-CD to the fermentation broth from 0·05 and 0·04 mM to 0·55 and 4·6 mM, respectively. In the absence of β-CD, only 0·4 mg/ml of lankacidin C accumulated at 48 h after the inoculation, the amount then decreased slowly during fermentation. In contrast, in the presence of β-CD, lankacidin C production continued throughout fermentation and finally reached 3·1 mg/ml. The production of lankacidinol, a minor by-product, was also stimulated by β-CD.

This stimulatory effect was observed for all *Streptomyces* species known to produce lankacidins. Beta-cyclodextrin had no marked effect on microbial growth, consumption rate of carbon source and pH changes throughout fermentation. It was not consumed by the microorganism during fermentation, and the lankacidins produced existed as inclusion complexes in the culture filtrate. It was found to be most effective around 1·5% (w/v) concentration (Fig. 6).

Dimethyl- and trimethyl-β-CD heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin; commercial name: TRIMEB inhibited lankacidin production,

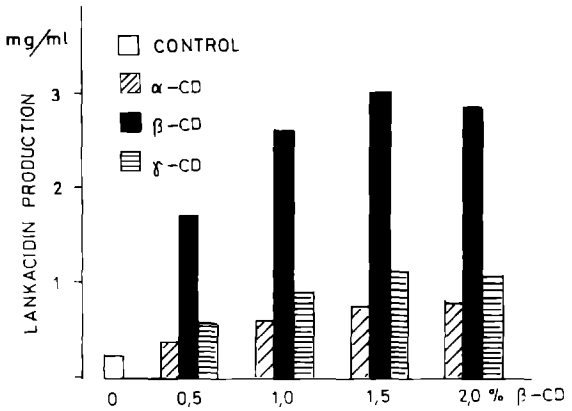

Fig. 6. Effect of CDs on lankacidin production (lankacidin C + lankacidinol) by *Streptomyces rochei* var. *volubilis* in five days fermentation (Sawada *et al.*, 1987).

because they remarkably inhibited the growth of the lankacidin producing microorganism.

Since the publication of these results, the effect of CDs have also been studied in other fermentations, e.g. a new anti-tumour substance, SPF-1000 is produced by growing a specific strain of Streptoccus; according to the patent application the culture media also contains CD (Shikishima Boseki, 1986).

ENHANCEMENT OF VACCINE PRODUCTION

Pertussis toxin (Leukocytosis promoting factor, LPF-hemaglutinin) is one of the main protective antigens against whooping cough infection, and one of the components of a pertussis vaccine. It is produced by *Bordatella pertussis*. For the production of a less reactogenic vaccine a synthetic medium is needed, but the production of pertussis toxin is rather difficult in synthetic media, especially in shaken cultures. *Bordatella pertussis* is very susceptible to a number of inhibitors, e.g. fatty acids (palmitic or oleic acid), even a 10 μM concentration stops the cell propagation (Suzuki *et al.*, 1983). However on adding 0·5 mg/ml dimethyl-β-CD (or trimethyl-β-CD) an increase in cell growth was observed (Table 3), moreover it enhanced the pertussis toxin production up to 100-fold (Imaizumi *et al.*, 1983*a,b*; Suzuki *et al.*, 1983*b*, 1984, 1985; Ginnaga *et al.*, 1984; Teijin Ltd, 1985). The mechanism of the effect is not yet fully understood, it could be sequestration of inhibitory fatty acids, stabilization of reduced glutathione, or the modification of the cell membrane permeability, etc. (Suzuki *et al.*, 1983*c*; Teijin Ltd, 1983). The enhance-

TABLE 3

Bordatella pertussis Cell Growth on CD-Containing Stainer–Scholte Medium as a Function of Inoculum Size (Imaizumi *et al.*, 1983*a*)

Inoculum cells in 5 μl	*Added CDs (0·5 mg/ml)*[a]				
	0	*α-*	*β-*	*γ-*	*DIMEB*
10^3	—	—	—	—	+ +
10^4	—	—	—	—	+ + +
10^5	—	—	—	—	+ + +
10^6	—	+ +	+	+	+ + +
10^7	—	+ + +	+ +	+ +	+ + +

[a]—, no growth; +, < 100 colonies; + +, 10^2–10^3 colonies; + + +, full growth.

ment of the production of filamentous haemagglutinin was even greater (several hundred times more was produced) in the presence of dimethyl-β-CD than without it (Suzuki *et al.*, 1984). In a 1000 litre industrial fermentor in Stainer–Scholte medium (which contained 1·67 g/liter dimethyl-β-CD and 0·1 g/litre methylcellulose) after 40 h the pertussis toxin level was 500, and the haemagglutinin level was 200 ELISA units/ml cell free medium (Quentin-Miller *et al.*, 1987). In clinical practice the diagnosis of whooping cough is not an easy task because *Bordatella pertussis* phase I is a finicky, fastidious, slow-growing bacterium that is difficult to isolate on laboratory media. Now a new solid dimethyl-β-CD containing medium, with a long shelf life has been reported, which overcomes the former problems (Aoyama *et al.*, 1986). Similarly CDs stimulated the in-vitro growth of *Mycobacterium lepraemurium* (Nakamura, 1982).

ENZYMIC REACTION OF LIPIDS

Hydrolysis of triglycerides by lipases in an aqueous system is a very slow process. Either a lipid dissolving water miscible organic solvent has to be added to the system, which is tolerated only to a relatively low concentration because of the enzyme–protein denaturation, or an appropriate detergent, e.g. natural bile has to be present. Table 4 illustrates the hydrolysis of olive oil by lipase (hog pancreas); without any detergent, in the presence of hog bile, or dimethyl-β-CD (Szejtli *et al.*, 1985). Following the hydrolysis the liberated fatty acids were titrated with NaOH. As can be seen, the dimethyl-β-CD remarkably accelerated the lipolysis. The observation that phosphatides (lignoceric acid, cerebroside, cera-

TABLE 4
Enzymic Hydrolysis of Olive Oil by Hog Pancreas

Reaction time (h)	*Consumed 0·005 M NaOH ml*		
	Control (no emulgeator)	*Hog bile added*	*Dimethyl-β-CD added*
0·5	0·0	0·82	1·74
1	0·36	1·17	2·00
2	0·48	1·37	2·20
19	0·63	2·62	4·77
Reaction rate acceleration		~4×	~7·4×

mide) can be solubilized with CDs will probably be exploited in lipid enzymology (Singh & Kishimoto, 1983).

Studying the lignoceryl-CoA ligase activity in a microsomal fraction prepared from rat brain, the lignoceric acid, solubilized by α-CD was utilized, but not the one solubilized by Triton WR 1339 (Singh *et al.*, 1985).

There are many diagnostic kits, which contain either a lipophylic substrate which then has to react with the appropriate enzyme of the biological sample, or an enzyme that has to react with a lipophylic component of the biological sample. In both cases the solubilizing capacity of CDs and particularly of substituted CDs, like dimethyl-β-CD can be well utilized. Examples are to be found in the literature dedicated to the use of CDs in diagnostics (Szejtli, 1988*a*, *b*).

The so-called secondary glycosides (digoxin, acetyl-digoxin and acetyl-digitoxin) of *Digitalis lanata* are important, highly active drugs, but the plant contains only small amounts of these substances, mainly the (primary) lanatoside glycosides, which have to be hydrolysed to obtain the secondary glycosides. Acid hydrolysis results in a high ratio of pharmacologically inactive by-products. The enzymic hydrolysis is more selective, but very slow because of the very low solubility of the substrate (solubility of lanatoside C in water = 0·18 mg/ml). Enhancing the solubility by adding ethanol or other water-miscible solvents to the water–lanatoside–enzyme suspension results in a large loss of enzyme activity. Dimethyl-β-CD as before enhanced the reaction rate, and moreover improved the selectivity (Nánási *et al.*, 1980, unpublished). Following the β-glucosidase catalysed hydrolysis in 10% dimethyl-β-CD containing solution by thin-layer chromatography only one product, the digoxin was formed (Fig. 7) with a yield of 50–60%, while in the presence of dioxane the yield was only 20–30%, besides a series of pharmac-

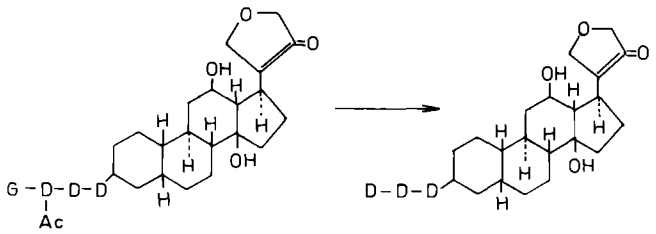

Fig. 7. Enzymic hydrolysis of lanatoside C to digoxin. D = D-digitoxose; G = D-glucopyranose; Ac = Acetyl group.

TABLE 5
Hydrolysis of Lanatoside C Glycoside by β-Glucosidase (isolated from *Penicillium)*

Reaction time (h)	*Lanatoside C dissolved in*			
	Dioxane		*DIMEB*	
	Lanatoside C (mg)	*Digoxin (mg)*	*Lanatoside C (mg)*	*Digoxin (mg)*
0	9·75	0·45	9·5	0·5
12	—[b]	—	9·0	1·0
24	9·45	0·5	7·6	1·8
36	—	—	5·75	3·2
48	8·05	0·69	4·5	3·9
96	8·35	1·35	2·75	6·4
144	7·35	1·7	1·95	7·6

[a]A 10 mg sample of Lanatoside C (isolated from *Digitalis lanata*) was dissolved in 2 ml dioxane or in 2 ml aqueous (10%) DIMEB solution (solubility of the substrate in this solution is 9·08 mg/ml, i.e. 50-fold better than in water), and added to the enzyme dissolved in 18 ml, pH 5·5, phosphate–citrate buffer, at room temperature.
[b]Not determined.

ologically inactive by-products. Certainly the steroid moiety is incorporated into the CD-cavity, and this steric orientation may explain the improved selectivity. Table 5 illustrates the reaction rate enhancement in the presence of dimethyl-β-CD (DIMEB). In trimethyl-β-CD solutions the rate increase was only about half of that of dimethyl-β-CD.

TISSUE CULTURES

The CD complexes of unsaturated fatty acids can be utilized as serum substitutes in mammalian cell structures. Both oleic acid- and linoleic

acid-β-CD complexes showed growth enhancing effects on human lymphoblast cells (up to 100 mg/litre medium). At higher concentration the fatty acid β-CD complex was found to be toxic, but this can probably be attributed to the fatty acids; 100 mg fatty acid-β-CD complex and 1000 mg free β-CD together resulted in no toxic effects, but exhibited a stable and reproducible growth promoting effect. In human diploid fibroblast cultures, growth similar to that in bovine albumin supplemented medium was observed after adding β-CD-fatty acid complex solution to a final concentration of 10–20 mg/ml. Bovine serum albumin can be partially or completely substituted by β-CD–fatty acid complexes in mammalian cell cultures (Yamane *et al.*, 1981) e.g. in human interferon production (Ajinomoto Co., 1982).

High density culture B-lymphoblast-like cell strains can be prepared in a serum-free medium which contains α-CD, *e.g.* in the case of the production of interferons from UMCL cells, the amount of interferons produced may be increased (Agency of Ind. Sci. Techn., 1986). Mouse mammary tumour cells can be cultured under serum-free conditions when the bovine albumin is substituted by α-CD complex of oleic acid (Agency of Ind. Sci. Tech., 1985; Kawamura *et al.*, 1985).

Beta-cyclodextrin is a useful material as a serum substitute in inducing primary antibody response *in vitro*. Fetal calf serum must be present at 10% in the culture medium for optically eliciting the primary antibody response to sheep erythrocytes in murine lymphocytes. The response cannot be observed when the concentration of the fetal calf serum is less than 1%. Addition of 250–500 μg/ml β-CD was the most effective; α-, γ-CD and dimethyl-β-CD were less effective (Ohmori & Yamamoto, 1987; Ohmori *et al.*, 1987*a*). Culture media, which contained β-CD as a substituent of fetal calf serum, was found to be useful for *in vitro* evaluation of immunomodulating agents (Ohmori *et al.*, 1987*a*) and in limphocyte cultures (Ohmori *et al.*, 1987*b*).

Alpha-cyclodextrin can be used as a cholesterol carrier in serum-free carrier, protein-free medium in tissue cultures, for example in the propagation of newborn rat adrenocortical cells. The α-CD forms a nearly 1 : 1 complex with cholesterol (Hammani *et al.*, 1986).

The use of α-CD in serum-free medium of mammalian cell cultures prolonged cell life, and increased the production of interferon and monoclonal antibody (Minamoto & Mitsugi, 1984). Besides the solubility enhancement, stabilization is the other important consequence of complexation of nystatin (a polyene antibiotic) with γ-CD. Nystatin is a frequently used antifungal antibiotic, widely used in human medicine for local antifungal treatment. Tissue culture, an important tool in biotechnology, needs soluble, antifungal agents, which have to be dissolved in

the culture medium. Nystatin would be very adequate for this purpose, however it is practically insoluble in water and rapidly decomposes by oxidation. The nystatin–γ-CD complex is a relatively stable, easily soluble powder, well applicable for such purposes (Szejtli *et al.*, 1983).

DETOXICATION OF INDUSTRIAL WASTEWATERS

One of the most effective and economic purification methods for industrial and domestic waste waters is biological oxidation by activated sludge systems. The biological waste water treatment means that these toxic substances are degraded by certain yeasts and bacteria which are present in the biological sludge. The waste waters of the food industries are generally liable to biological degradation, but those of the organic chemical industry, for example pesticides, drugs, their intermediaries, etc., may be more or less resistant to biological degradation. These chemicals can be tolerated and metabolized by the microbial flora of the activated sludge system only up to certain levels. On surpassing the toxic concentration tolerance level the microbial flora is damaged, and the biological activity of the sludge decreases more or less irreversibly.

To avoid damage, either the concentration of toxic substances has to be held below the tolerance level, e.g. by diluting it with toxic substance-free water, or the toxicity threshold of the microbes has to be enhanced by appropriate adaptation processes. Unambiguously, both possibilities are limited by technical and biological factors. A third alternative is the partial and temporary masking of the toxic substances by converting them to non-toxic CD inclusion complexes (Farkas *et al.*, 1986; Oláh *et al.*, 1988). Of course, the use of CDs is restricted to CD-complex-forming organic toxic substances.

The probable explanation for the toxicity reducing effect of β-CD is the reduced affinity of the relatively large hydrophobic complexes to the cell membranes. There is another possible explanation, in which the complexed guest molecules in the CD cavity are more liable to biological decomposition than in free, non-complexed form, resulting in their more rapid elimination from the activated sludge system. This is however unlikely, because in the initial phase the metabolism of the toxic substances is slower (see Table 6) proving that the complexes are metabolized at a reduced rate. Therefore the crucial factor is in the protection of the living cells through the reduction of the concentration of the toxic-free molecules. This is illustrated on the detoxication of a pharmaceutical waste water (Fig. 8).

In the presence of toxic substances the acetate metabolizing capacity of the activated sludge decreases, the extent of inhibition increases with

TABLE 6
Effect of β-CD on the Decomposition of Some Chlorophenols in Activated Sludge System

Sampling time	*Compound*	*Decomposition (%)*	
		Without β-CD	*With β-CD*
2 h	*p*-Chlorophenol	23·3	9·5
4 h		25·9	53·4
6 h		27·6	54·4
1 day	2,4-Dichlorophenol	7·5	1·3
2 days		25·4	18·5
4 days		26·0	32·1
7 days		29·5	73·0
1 day	Penta-chlorophenol	0·7	0·2
2 days		20·4	1·7
4 days		23·3	0·3
7 days		56·7	2·5

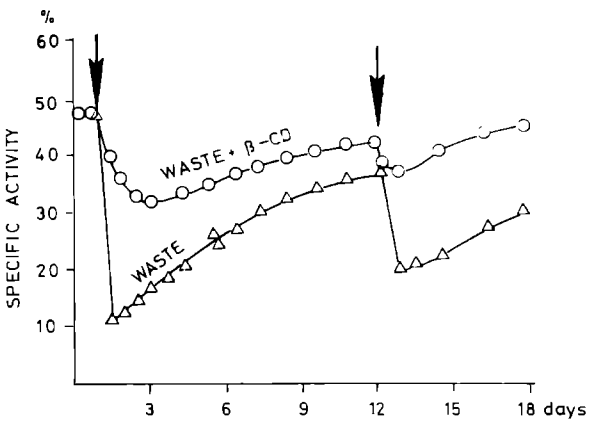

Fig. 8. The effect of β-CD on the adaptation of activated sludge to pharmaceutical waste. Activity of blank sludge sample: 47 mg acetate COD (Chemical Oxygen Demande)/gh. The weight ratio of β-CD to waste COD = 0·5 : 1; ↓ = time of feed; ○ = activity values at 200 mg/lCOD + 100 mg/lβ-CD; △ = activity values at 200 mg/l waste without β-CD.

increasing toxic substance concentration. Adding the toxic chemicals containing pharmaceutical waste water to the activated sludge, the acetate metabolizing capacity immediately drops back, and only slowly regenerates. If however β-CD is simultaneously added, the drop in activity (acetate metabolizing capacity) is much smaller, and within some days its value is again near to the original one. The second addition of the

toxic substance causes an even smaller effect in the CD-containing sludge. Either the microbial flora is better adapted to the toxic substances in the presence of β-CD or the β-CD having a lower decomposition rate than the complexed toxicant remains for a longer time in the activated sludge, protecting it from toxic effects and maintaining its detoxicating capacity (Oláh *et al.*, 1988). The principle of the practical utilization of this possibility is illustrated in Fig. 9.

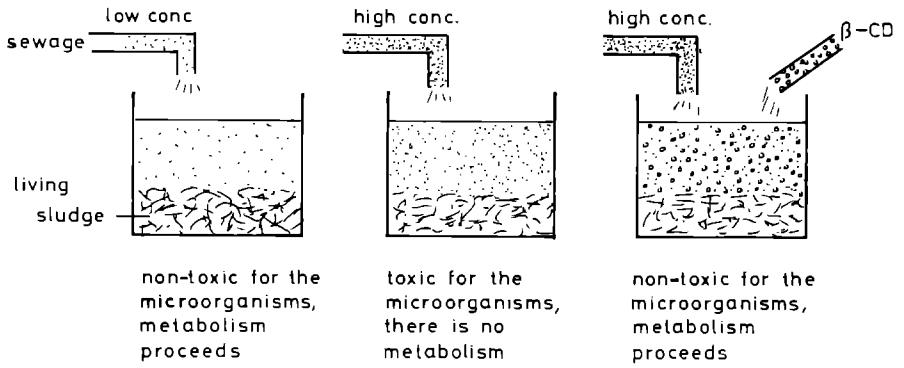

Fig. 9. The tolerable level of the toxic compounds is augmented by adding CD to the toxic sewage.

The detoxication in the presence of β-CD may sometimes get strongly decelerated, as is seen in Table 6. In contrast to the *para*- and 2,4-dichlorophenols, the β-CD complexation inhibits the decomposition of pentachlorophenol. This is probably due to the fact that the bulkier *penta*-chlorophenol molecule forms an extremely stable complex with β-CD. This complexation may protect the OH group from enzymatic attack.

Not only natural CDs but also some derivatives can be used in such detoxications. The addition of soluble β-CD-polymer to phenol containing sewage increases the efficiency of microbiological detoxication of phenol by *Candida tropicalis*. The cells fixed in the polymer beds together with the β-CD-polymer can be reused for sewage treatment (Bánky *et al.*, 1985).

REFERENCES

Agency of Ind. Sci. Tech. (1985) Jpn. Kokai (Patent no.) 85, 87, 785.
Agency of Ind. Sci. Tech. (1986) Jpn. Kokai (Patent no.) 86, 63, 279.

Ajinomoto Co. (1982) Jpn. Kokai, 82, 194, 787, (C.A. 98:124208).
Aoyama, T., Murase, Y., Iwata, T., Imaizumi, A., Suzuki, Y. & Sato, Y. (1986). *J. Clin. Microbiol.*, **23,** 1046.
Bánky, B., Recseg, K. & Novák, B. (1985). *Magy. Kém. Lapja (Budapest),* **40,** 188.
Bar, R. (1989). *Trends in Biotechnol.*, **7,** 2.
Bender, M. L. & Komiyama, M. (1978). *Cyclodextrin Chemistry.* Springer-Verlag, Berlin.
Bergeron, A. R., Machida, Y. & Bloch, K. (1975). *J. Biol. Chem.*, **250,** 1223.
Duchéne, D., ed. (1987). *Cyclodextrins and their Industrial Use.* Editions de Sante, Paris.
Farkas, P., Szente, L., Oláh, J., Szejtli, J. & Cserháti, T. (1986). Hung. Teljes (Patent no.) HU 37, 727 (C.A. 105: 84597).
Ginnaga, A., Koba, H., Sakuma, S., Kitagawa, H., Yamada, A. & Suzuki, Y. (1984). European Patent Application EP 121249 (C.A. 102: 4360).
Hammani, M., Maume, G. & Maume, B. (1986). *Cell Biol. Toxicol.*, **2,** 41. (C.A. 105: 219064).
Hesselink, P. G. M., de Vries, H. & Witholt, B. (1987). In *Proceedings of the 4th European Congress on Biotechnology,* Vol. 2, ed. O. M. Neijssel, R. R. van der Meer & K. C. A. M. Luyben. Elsevier, Amsterdam, p. 299.
Imaizumi, A., Suzuki, Y., Ono, S., Sato, H. & Sato, Y. (1983*a*). *Infection and Immun.*, **41,** 1138.
Imaizumi, A., Suzuki, Y., Ono, S., Sato, H. & Sato, Y. (1983*b*). *J. Clin. Microbiol.*, **17,** 781.
Kawamura, K., Enami, J., Kohmoto, K., Koga, M. (1985). *Dokkyo J. Med. Sci.*, **12,** 167, (C.A. 104: 164689).
Machida, Y., Bergeron, R., Flick, P. & Bloch, K. J. (1973). *J. Biol. Chem.*, **248,** 6246.
Minamoto, Y. & Mitsugi, K. (1984). Growth of Differentiated Cells in Defined Environments In Proceedings of the International Symposium, ed. H. Murakami. Kodansha Tokyo, p. 127.
Nakamura, M. (1982). *Nippon Rai Gakkai Zasshi,* **51,** 81.
Ohmori, H. & Yamamoto, I. (1987). *J. Immun.*, **17,** 79: (C.A. 106: 174219).
Ohmori, H., Ienaga, M. & Yamamoto, I. (1987*a*). *Jpn. J. Pharmacol.*, **44,** 225. (C.A. 107: 51288).
Ohmori, H., Ienaga, M. & Yamamoto, I. (1987*b*). *J. Pharmacobiol-Dyn.*, **10,** 384. (C.A. 107: 196150).
Oláh, J., Cserháti, T. & Szejtli, J. (1988). *Water Res.*, **22,** 1345.
Quentin-Millet, M. J. B. J., Arminjon, F. & Donikian, R. R. (1987). European Patent Application EP 239, 504 (C.A. 108: 36399).
Sawada, H., Suzuki, T., Akiyama, S., Nakao, Y. (1987). *Appl. Microbiol. Biotechnol.*, **26,** 522. (C.A. 107: 196443).
Shikishima Boseki (1986). Jpn. Kokai (Patent no.) 85, 239, 424.
Singh, I. & Kishimoto, Y. (1983). *J. Lipid Res.*, **24,** 662.
Singh, I., Singh, R., Bhushan, A. & Singh, A. K. (1985). *Arch. Biochem. Biophys.*, **236,** 418. (C.A. 102: 91817).
Suzuki, I., Okada, J. & Sawada, H. (1983*a*). (Takeda Chemical Ind.) European Patent Application EP 91 781, (C.A. 100: 4792).
Suzuki, Y., Imaizumi, A., Sato, M. & Sato, Y. (1983*b*). *Jpn. J. Med. S.*, **36,** 111.

Suzuki, Y., Imaizumi, A., Yamaguchi, H., Kanesaki, M., Ono, S. (1983*c*) Jpn. Kokai 83, 67, 182 (C.A. 33: 138191).
Suzuki, Y., Imaizumi, A., Ono, S., Sato, H. & Sato, Y. (1984). *Abstract Book of 3rd Int. Symp. Clathrate Comp. and 2nd Int. Symp. on Cyclodextrins,* ed. P. Nagai. Hoshi University, Tokyo, pp. 131–2.
Suzuki, Y., Imaizumi, A., Ono, S. & Sato, Y. (1985). *J. Pharmacobio-Dyn.,* **8,** 39.
Szejtli, J. (1982). *Cyclodextrins and their Inclusion Complexes.* Akadémiai Kiadó, Budapest.
Szejtli, J. (1986). Starch/Stärke, **38,** 388.
Szejtli, J. (1988*a*). *Cyclodextrin Technology,* Kluwer Academic Publishers, Dordrecht.
Szejtli, J. (1988*b*). *Kontakte,* **1,** 31.
Szejtli, J., Stadler-Szõke, Á., Vikmon, Á., Piukovich, S., Inczefy, I., Kulcsár, G. & Zlatos, G. (1983). Hung. Teljes (Patent no.) HU 35, 172 (C.A. 103: 200887).
Szejtli, J., Szente, L., Kálói, K., Marton, J. & Gerlóczy, A. (1985). Hungarian Patent Application 75/85.
Teijin Ltd. (1983) Jpn. Kokai (Patent no.) 83, 67, 188, (C.A. 99: 193225).
Teijin Ltd. (1985) Jpn. Kokai (Patent no.) 85, 155, 127.
Udvardy, N. E., Bartha, I., Hantos, G., Trinn, M., Vida, Zs., Szejtli, J., Stadler-Szõke, Á., Habon, I. & Balázs, M. (Richter Gedeon) (1983) Belgian Patent 894 501 (C.A. 99: 4069).
Uekama, K., Irie, T., Sunada, M., Otagiri, M., Iwasaki, K., Okano, Y., Miyata, T. & Kase, Y. (1981). *J. Pharm. Pharmacol.,* **33,** 707 (C.A. 96: 135691).
Yamane, I., Kan, M., Minamoto, Y. & Amatsuji, Y. (1981). Proc. Jpn. Acad., Ser., and (1982) Cold Spring Harbor Conf. Cell Proliferation p. 87 (C.A. 97: 212006).