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Dual Behaviour of Maltosyl-β-Cyclodextrins: Investigation of Formation of Inclusion and Glucose-type Complexes

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Abstract. To clarify the complexation behaviour of the less studied maltosyl-β-cyclodextrin, its interactions with some characteristic guests such as phenolphthalein and *p*-nitrophenol have been studied. The results show that similar types of inclusion complexes are formed as in the case of the unsubstituted β-cyclodextrin. In addition to the formation of inclusion complexes, maltosyl-β-cyclodextrins interact with other complexing reagents, like borate, vanadate or molybdate and the maltosyl substituent forms sugar-type complexes. The properties of these complexes are similar to those of glucose.

Key words: maltosyl-β-cyclodextrin, inclusion complexes, formation constants, sugar-type complexes, induced circular dichroism spectroscopy

1. Introduction

Cyclodextrins (CDs) are cyclic oligosacharides which are produced by enzymatic hydrolysis of starch [1–5]. The most common CDs are α, β and γ-CD containing six, seven or eight α-D-glucopyranose units, respectively. They are molecules with a shape of a truncated cone having a hydrophobic cavity. Many hydroxy groups are situated on the outer part of the ring which make CDs both hydrophilic and water soluble. It is well known that CDs can form inclusion complexes with guest molecules which might change the properties of the encapsulated molecule (increasing its water solubility, chemical stability, etc.). CDs have a wide range of practical applications in pharmaceutical, cosmetic, food, chemical and several other industries [1–5]. They are useful in many of the separation techniques, being chiral selectors. Structurally similar compounds, isomers and even enantiomers can be separated [4–7]. Several CD derivatives have been synthesised [5] to improve the hydrophobic character of the cavity and/or to change the solubility in water or in different organic solvents.

Maltosyl-β-CDs (Figure 1) contain one or more maltosyl side chains and they belong to the second generation of CDs. These compounds can be considered as the "most natural" derivatives of the parent β-CD. Their first preparation was

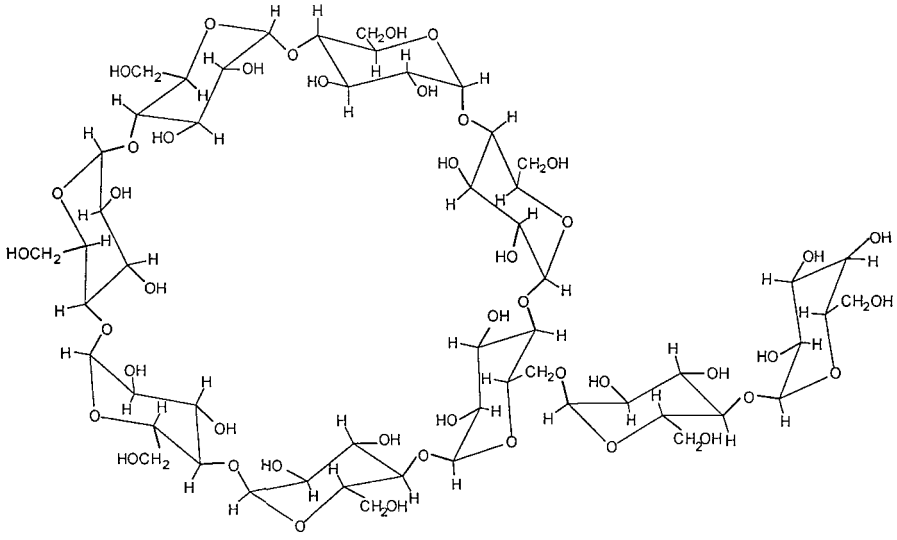

Figure 1. Atom-and-bond representation of (mono)maltosyl-β-CD.

reported in 1965 [8]. Nowadays, maltosyl-β-CD is produced from starch using pullulanase (pullulane-6-glucanohydrolase) [8–11] and isoamylase (glycogen-6-glucanohydrolase) [12–14]. Other methods use maltosyl fluoride and β-CD with pullulanase [15–16]. Despite the fact that advantageous characteristics have been recognized, the different interactions of maltosyl-β-CD (such as inclusion complex formation) have hardly been investigated. The chemical properties of the sugar type [maltosyl = α-D-gluco-pyranosyl(1 $\rightarrow$ 4)-O-α-D-glucopyranosyl(1 $\rightarrow$ {2,3,6}^{A})-] side chain is almost unknown.

As maltosyl-β-CD consists of two significant parts such as the CD ring and the maltosyl side chain (Figure 1), it is expected to show similar complex forming properties as its constituents. Our aim was to investigate and to compare maltosyl-β-CD with β-CD and glucose based on their complex formations.

2. Experimental

Maltosyl-β-CD was a gift of Ensuiko Sugar Refining Company Ltd. (Yokohama, Japan) and was used without further purification. The amount of adsorbed water was measured as the drying loss at 110 °C for 2 hours. Phenolphthalein (PP) and glucose were recrystallized from ethanol-water mixtures. *p*-Nitrophenol (*p*-NP) was of analytical grade and no impurities could be detected. Borax [sodium tetraborate, $Na_2B_4O_5(OH)_4.8H_2O$], sodium vanadate and molybdate as well as all buffer materials were of analytical grade.

Mass spectra were recorded on a ZAB-2SEQ spectrometer using the FAB(+) method. Induced circular dichroism (ICD) spectra were measured using a Jobin-Yvon Dichrograph Mark VI. The concentration of PP was 2.5×10^{-5} M, and the

maltosyl-β-CD was in relatively large excess (8×10^{-3} M) to ensure almost full complexation in the solution. The temperature was kept constant at 25 ± 1 °C.

Spectrophotometric measurements were carried out with a Spectromom 195D instrument in two series at different wavelengths: (i) at 550 nm for PP [17] and glucose/PP systems; and (ii) at 300 and 402 nm (absorption maxima of the protonated and deprotonated *p*-NP) and at 317 nm (their isosbestic point) [18]. In series (i), the concentration of PP was 3×10^{-5} M and that of the maltosyl-β-CD was 1.5×10^{-5}–3.0×10^{-4} M, while a large excess of glucose was used (up to 1.0 M). The pH and ionic strength was adjusted by 0.02 M Na_2CO_3. In series (ii), the concentration of *p*-NP was 5×10^{-5} and/or 1×10^{-4} M, that of maltosyl-β-CD varied from zero to 1.0×10^{-2} M. The pH was adjusted around the pK_A value (6.90) of *p*-NP: the actual values were 6.8, 7.0 and 7.2. Ionic strength was kept constant in each measurement (I = 0.2 M). The molar absorptivities of protonated and deprotonated *p*-NP were determined at pH = 2.0 and at pH = 12.0 at different wavelengths.

Potentiometric titrations were carried out using a Radiometer pHM93 instrument with a pHC2406 glass electrode. The ionic strength was 0.02 and 1.00 M Na(Cl), respectively, and the temperature was kept strictly constant at 25.0 ± 0.1 °C. As the best method [19], 2.00 mL aliquot parts of 2.65×10^{-3} M sodium tetraborate were titrated with 1.75×10^{-2} M maltosyl-β-CD. The electrode was calibrated with standard buffer solutions as regular, and a Radiometer ABU-12 automatic burette was used for fine volume addition.

Optical rotations were measured using a Zeiss Polamat A multiwavelength polarimeter. The final concentration of maltosyl-β-CD was 5×10^{-3} M, that of β-CD and glucose, used for comparison was identical. Molybdate and vanadate, respectively, were also used in this (equimolar) concentration. The solutions were made acidic with 0.2 M formic acid, and the temperature was kept constant at 25.0 ± 0.1 °C.

3. Results and Discussion

As the enzymatic degradation does not usually lead to formation of pure products, the sample investigated must be assumed to be a mixture of CDs of different side chains (e.g., glucosyl, maltosyl) and with different degrees of substitution. Mass spectroscopic measurement showed (Figure 2), that the maltosylation gives a heterogeneous product: peaks with masses of 1784, 1622, 1460 and 1297 can be identified as dimaltosyl-β-CD [$(G_2)_2\beta$CD], maltotriosyl-β-CD [$G_3\beta$CD] or monomaltosyl-monoglucosyl-β-CD [$G_2G\beta$CD], monomaltosyl-β-CD [$G_2\beta$CD] or diglucosyl-β-CD [$(G)_2\beta$CD], and monoglucosyl-β-CD [GβCD], respectively. A small peak of 20% intensity with mass 1135 seems to represent the unsubstituted β-CD. The spectrum gives clear evidence – as with most natural or half-synthetic materials – for the multicomponent character of the sample. Because fragmentation might be possible under the condition of the measurements, comparison

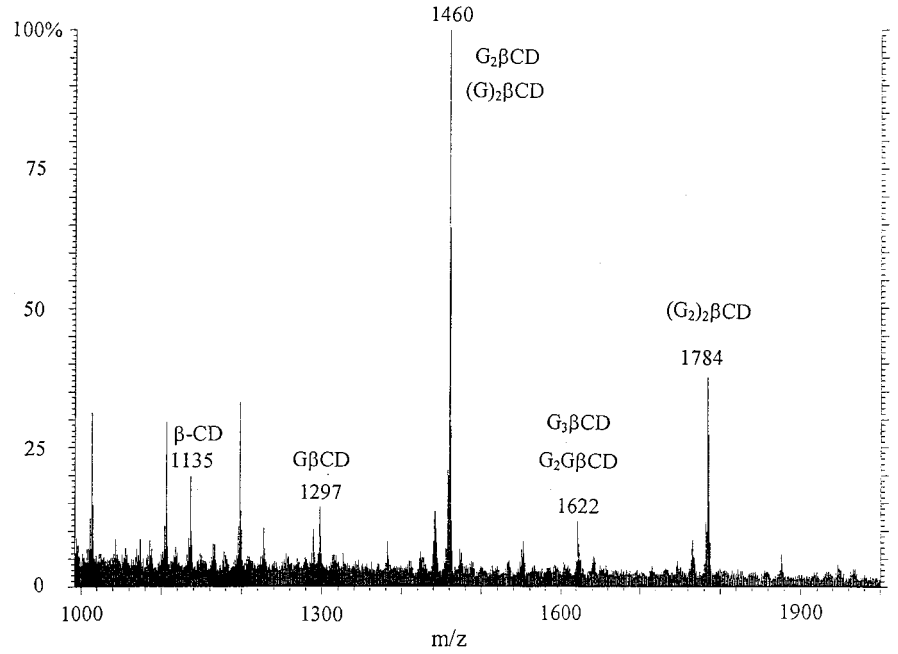

Figure 2. A characteristic part of the mass spectrum of the maltosyl-β-CD sample investigated.

of peak heights cannot be used to quantify the ratios of different CDs. [e.g., the real ratio of $G_2\beta$CD and $(G_2)_2\beta$CD is expected to be smaller than the peak ratio calculated from the spectrum.] Qualitative and quantitative composition of this maltosyl-β-CD sample was determined on the basis of the HPLC chromatogram kindly provided by Ensuiko Sugar Refining Company Ltd [20]. Accordingly, the air-dried sample contains nearly a 1:1 mixture of $G_2\beta$CD and $(G_2)_2\beta$CD (40.8% and 45.7%), while the amount of unsubstituted β-CD is as small as 0.9%. Using this result, the average molar mass could be calculated.

As mentioned, the inclusion complex formation properties of maltosyl-β-CD were intended to be studied with different guest molecules and to compare the findings to that of the parent β-CD. As a first step, the interaction between glucose and β-CD had to be investigated, since it has been reported recently – in contrast to earlier results [21] – that a rather stable complex is formed [22]. The existence of such a complex would make difficulties in characterizing complexation equilibria in the system because the glucose type α-maltosyl side chain could interact with the CD cavity via self-inclusion, competing with any further interactions and producing maltosyl-β-CD aggregates. From competitive spectrophotometric measurements [23] we concluded (in agreement with other authors [24]), that there is no significant interaction between glucose and β-CD ($\beta_{11} \leq 10^{-2}$). From this fact, the self-association of maltosyl-β-CD can be excluded.

Table I. Stability constants of different complexes formed with maltosyl-β-CD

System[a]	β_{11} (M^{-1})	Medium	T (°C)	Ref.
β-CD + PP	$(2.30 \pm 0.15) \times 10^4$	0.02 M Na_2CO_3	25	17
Maltosyl-β-CD + PP	$(3.20 \pm 0.20) \times 10^4$	0.02 M Na_2CO_3	25	This work
β-CD + *p*-NP	$(1.30 \pm 0.15) \times 10^2$	0.2 M NaCl	25	18
Maltosyl-β-CD + *p*-NP	$(2.00 \pm 0.25) \times 10^2$	0.2 M NaCl	25	This work
β-CD + *p*-NPate$^-$	$(4.10 \pm 0.40) \times 10^2$	0.2 M NaCl	25	18
Maltosyl-β-CD + *p*-NPate$^-$	$(4.60 \pm 0.50) \times 10^2$	0.2 M NaCl	25	This work
β-CD + benzoate	$(5.00 \pm 0.90) \times 10^1$	0.1 M NaCl	25	31
G_2-β-CD + benzoate	$(1.25 \pm 0.09) \times 10^2$	0.1 M buffer[b]	28	27
$(G_2)_2$-β-CD + benzoate	$(1.52 \pm 0.13) \times 10^2$	0.1 M buffer[b]	28	27
D-glucose + β-CD	$(4.20 \pm 0.05) \times 10^2$	$\sim$0	25	22
D-glucose + β-CD	$\leq 10^{-2}$	0.02 M Na_2CO_3	25	This work
D-glucose + H_3BO_3	$(4.20 \pm 0.55) \times 10^1$	0.015 M $NaClO_4$	25	19
Maltosyl-β-CD + H_3BO_3	$(3.50 \pm 0.40) \times 10^1$	0.02 M NaCl	25	This work
Maltosyl-β-CD + H_3BO_3	$(3.00 \pm 0.40) \times 10^1$	1.00 M NaCl	25	This work

[a] For abbreviations see text.
[b] Buffer = 0.1 M benzoic acid + TRIS [tris(hydroxymethyl)-aminomethane] in small excess (to pH = 7.0).

The purple colour of phenolphthalein (PP) in alkaline media (pH $\cong$ 10.5) is continuously faded with the addition of maltosyl-β-CD. As the molar absorbance for the complexed form at 550 nm is really zero, the stability constant of the existing 1:1 inclusion complex:

$$\beta_{\text{PP–CD}} = [\text{PP–CD}]/([\text{CD}] \times [\text{PP}]) \tag{1}$$

(where square brackets denote equilibrium concentrations) could be simply determined [23]. (The actual β values are summarized in Table 1.) Comparing the calculated value for the inclusion complex of PP and maltosyl-β-CD with that of unsubstituted β-CD, a significant increment in complex stability can be noticed which suggests the contribution of the maltosyl side chain in the interaction, presumably by H-bonds.

Induced circular dirchroism (ICD) spectra (Figure 3) clearly show that in addition to the stability of the PP complexes their structure is also similar. Around neutral conditions the peak maxima and their signs show correlation, and in addition to this, at pH = 10.5 the peak heights for the maltosyl-β-CD complex are in good agreement with that of β-CD [17]. After the theory of the coupled oscillator model [25, 26], the sign of the ICD is influenced not only by the direction but also by the depth of the insertion of the chromophore. This means that the angle between the PP and the CD ring is almost identical and the penetration of the PP quinone part has a similar pattern. It follows that the three-site interaction which

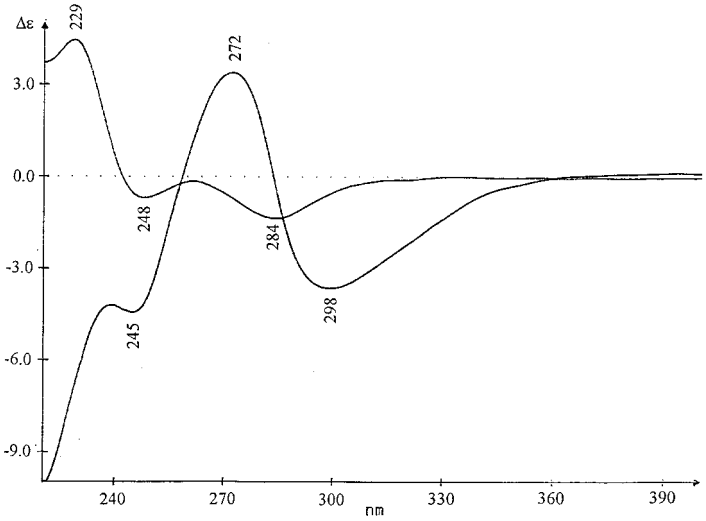

Figure 3. Induced circular dichroism spectra of phenolphthalein – maltosyl-β-CD inclusion complex. [Upper curve (at the beginning): in neutral solution; lower curve (at the beginning): at pH = 10.5.]

is characteristic in molecular recognition of PP by β-CD [17] is realized also by maltosyl-β-CD, moreover it seems to be promoted by the (H-bonded) participation of maltosyl substituent(s).

The investigation of *p*-nitrophenol (*p*-NP) complexes of maltosyl-β-CD was based on the shift of the protonation equilibria in the presence of CD since the stabilities of CD complexes with *p*-NP and *p*-NPate are different. Uv-vis spectra of *p*-NP and its deprotonated form differ significantly while complexation results in only minor changes [18]. For evaluation of the different constants, the following equations were used:

$$c_{\mathrm{NP}} = [\mathrm{NP}] + [\mathrm{NPate}^-] + [\mathrm{CD{-}NP}] + [\mathrm{CD{-}NPate}^-] \tag{2}$$

$$c_{\mathrm{CD}} = [\mathrm{CD}] + [\mathrm{CD{-}NP}] + [\mathrm{CD{-}NPate}^-] \tag{3}$$

where c_{NP} and c_{CD} represents the total concentrations of *p*-NP and maltosyl-β-CD; NP, NPate^-, CD–NP, $\mathrm{CD{-}NPate}^-$ are *p*-NP, its anion and their CD complexes, respectively, and [ ] denotes equilibrium concentrations. At each of three wavelengths, all species contribute with their own molar absorptivities to the total absorption characterized as follows:

$$A_i = \epsilon^i_{\mathrm{NP}}[\mathrm{NP}] + \epsilon^i_{\mathrm{NPate}}[\mathrm{NPate}^-] + \epsilon_{\mathrm{CD-NP}}[\mathrm{CD\text{-}NP}] + \epsilon_{\mathrm{CD-NPate}}[\mathrm{CD\text{-}NPate}^-] \tag{4}$$

where ϵ^i is the molar absorptivity of the given species at the ith wavelength. Substituting equations of complex formation and the protonation constants of *p*-NP

in Equations (2)–(4), the number of variables can be decreased. The constants of ϵ^i_{NP} and ϵ^i_{NPate}- can be determined in pure acidic and alkaline solutions of p-NP separately, while ϵ^i_{CD-NP}, $\epsilon^i_{CD-NPate}$- and the complex formation constants (β_{CD-NP}, $\beta_{CD-NPate}$-):

$$\beta_{CD-NP} = [CD–NP]/([CD] \times [NP]) \tag{5}$$

$$\beta_{CD-NPate}\text{-} = [CD–NPate^-]/([CD] \times [NPate^-]) \tag{6}$$

were varied by an iterative computer program. (The β values calculated can also be seen in Table 1.) The results with maltosyl-β-CD show that p-NPate forms a more stable complex than the undissociated p-NP and follows the trend experienced with β-CD [18]. It is interesting to observe that just as in the case of PP complexation, the formation constants of maltosyl-β-CD complexes have also higher values than the unsubstituted β-CD complexes.

It is worth mentioning that the benzoate anion also gives more stable complexes with maltosyl-β-CD(s) than with β-CD, as measured by the HPCE method [27], and in addition the separate formation constants for benzoate inclusion complexes with mono- and dimaltosyl-β-CDs could be calculated. (They are also given in Table 1 for comparison.)

The α-maltosyl side chain connected to the β-CD ring (Figure 1) adds new and different chemical characteristics to the properties of parent β-CD. The α-maltosyl group, like the free maltose (or glucose) bears a reducing end: the cyclically bonded glycosidic hydroxy group is able to open and the free end group can be oxidised. So it is not surprising that maltosyl-β-CD reacts with periodate in a Malaprade type reaction (in slightly acidic solution) or with iodine (in alkaline media) nearly stoichiometrically, as proved by our preliminary investigations. (β-CD does not react similarly since all of its glycosidic hydroxy groups are bound.) These reactions must be connected to the sugar type behaviour of maltosyl-β-CD.

Similarly, the interactions of maltosyl-β-CD with molybdate or vanadate ions in acidic solutions produce consequences (e.g., a significant large change in optical rotation) characteristic for glucose and nonexistent in the case of native β-CD.

It is well known that D-glucose forms complexes with borate anions and NMR investigations proved that the D-glucose in the complex is in the furanose form [28, 29]. As the complex formation between boric acid and polyhydric compounds reduces the hydroxide ion concentration in aqueous solution, pH-potentiometric titration of tetraborate solution with maltosyl-β-CD was carried out and the increase in pH was measured [19]. The background is that borax (in diluted aqueous solution) is in a well-known equilibrium with boric acid and hydroxide ion (and those with each other), as follows:

$$B_4O_5(OH)_4^{2-} + 5H_2O \Leftrightarrow 4H_3BO_3 + 2OH^- \tag{7}$$

$$K_B = ([H_3BO_3] \times [OH^-])/[B(OH)_4^-] \tag{8}$$

The value of K_B was calculated from the initial pH for each ionic strength. Since borax solution contains a conjugate acid-base pair [Equation (7)], it behaves as a stable buffer: the effect of dilution itself did not cause a change in pH. During the titration, the tetrahydroxo borate concentration is decreased by the added maltosyl-β-CD solution as the maltosyl-β-CD–borate complex is formed:

$$\beta_{\text{CD–borate}} = [\text{CD–B(OH)}_4^-]/([\text{B(OH)}_4^-] \times [\text{CD}]) \quad (9)$$

(where CD denotes strictly maltosyl-β-CD and not β-CD). Since $B(OH)_4^-$ plays both the central role in equilibria (8) and (9), the formation constant of the CD–$B(OH)_4^-$ complex can be determined from the pH-titration curve (Table 1). [To adjust ionic strength, NaCl was used instead of $NaClO_4$ since the perchlorate anion forms relative stable complex(es) with CD [30] and influences the potentiometric measurement.] It can be clearly seen that maltosyl-β-CD forms borate complexes with similar stability to the D-glucose complexes. This indicates that in this system the CD ring can be considered as a big "substituent" which does not influence the complex formation.

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