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Carbohydrate Research 341 (2006) 210–217
Carbohydrate RESEARCH

Optimized synthesis of specific sizes of maltodextrin glycosides by the coupling reactions of *Bacillus macerans* cyclomaltodextrin glucanyltransferase

Seung-Heon Yoon and John F. Robyt*

Laboratory of Carbohydrate Chemistry and Enzymology, Department of Biochemistry, Biophysics, and Molecular Biology, 4252 Molecular Biology Building, Iowa State University, Ames, IA 50011, USA

Received 19 August 2005; accepted 4 November 2005
Available online 2 December 2005

Abstract—*Bacillus macerans* cyclomaltodextrin glucanyltransferase (CGTase, EC 2.4.1.19), in reaction with cyclomaltohexaose and methyl α-D-glucopyranoside, methyl β-D-glucopyranoside, phenyl α-D-glucopyranoside, and phenyl β-D-glucopyranoside gave four kinds of maltodextrin glycosides. The reactions were optimized by using different ratios of the individual D-glucopyranosides to cyclomaltohexaose, from 0.5 to 5.0, to obtain the maximum molar percent yields of products, which were from 68.3% to 78.6%, depending on the particular D-glucopyranoside, and also to obtain different maltodextrin chain lengths. The lower ratios of 0.5–1.0 gave a wide range of sizes from d.p. 2–17 and higher. As the molar ratio was increased from 1.0 to 3.0, the larger sizes, d.p. 9–17, decreased, and the small and intermediate sizes, d.p. 2–8, increased; as the molar ratios were increased further from 3.0 to 5.0, the large sizes completely disappeared, the intermediate sizes, d.p. 4–8, decreased, and the small sizes, d.p. 2 and 3 became predominant. A comparison is made with the synthesis of maltodextrins by the reaction of CGTase with different molar ratios of D-glucose to cyclomaltohexaose.

Keywords: Synthesis of maltodextrin glycosides; Cyclomaltodextrin glucanyltransferase; Cyclomaltohexaose; Methyl α-D-glucopyranoside; Methyl β-D-glucopyranoside; Phenyl α-D-glucopyranoside; Phenyl β-D-glucopyranoside; Transglycosylation reactions; Acceptor reactions; Coupling reactions; Reaction optimization

1. Introduction

Bacillus macerans cyclomaltodextrin glucanyltransferase (CGTase) [EC 2.4.1.19] catalyzes three kinds of reactions: (1) formation of α-(1→4)-linked cyclomaltodextrins, containing primarily six D-glucopyranose units, CD6, but also forming cyclomaltodextrins containing seven and eight D-glucose units (CD7 and CD8) from starch;[1,2] (2) 'coupling' or 'acceptor' reactions, primarily between cyclomaltohexaose (CD6) and various carbohydrate acceptors, such as D-glucose, maltose, sucrose, and so forth;[3] and (3) disproportionation reactions that take place between two maltodextrin molecules that are formed in the coupling reaction to give a series of maltodextrins of different sizes.[1,4–6] In some instances, a fourth hydrolytic reaction can occur when water acts as an acceptor. This hydrolytic reaction had to have occurred when *B. macerans* CGTase reacted with CD6 to give a series of cyclomaltodextrins of d.p. 7–11 and maltodextrins d.p. 5–12 D-glucose residues.[7] In the presence of good acceptors, such as, D-glucose or methyl α-D-glucopyranoside, the reaction of water with *B. macerans* CGTase is much less favorable and the hydrolysis reaction is very minor, if it occurs at all.[7] More recently, a CGTase from *B. circulans* was shown to have a specificity for primarily forming CD7, with lesser amounts of CD6 and CD8.[8] Kinetic studies with *B. circulans* CGTase have shown that the enzyme has a specificity for catalyzing the coupling reaction 13.7-times faster using CD7 than using CD6,[9] in contrast with the

* Corresponding author. Tel.: +1 515 294 1964; fax: +1 515 294 0453; e-mail: jrobyt@iastate.edu

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doi:10.1016/j.carres.2005.11.014

coupling reaction catalyzed by *B. macerans* CGTase, which almost exclusively uses CD6 as the donor.[7]

B. macerans CGTase catalyzed coupling reactions have been used: to specifically synthesize ^{14}C-reducing-end labeled maltodextrins;[1,6,10] to synthesize methyl α- and β-maltodextrin glycosides;[11] to synthesize maltodextrin chains attached to the non-reducing end of acarbose;[12] to synthesize glycosylated salicyl alcohol to give salicin analogues;[13] and to synthesize higher cyclomaltodextrins containing 9–25 D-glucopyranose units.[7] *B. circulans* CGTase has also been used to synthesize maltodextrins.[14] Previously, we reported on the qualitative and quantitative distribution of maltodextrins and cyclomaltodextrin products formed in the coupling reaction of *B. macerans* CGTase between different molar ratios of D-glucose and cyclomaltohexaose.[7] The kinds of products formed and their quantitative amounts varied, depending on the ratios and concentrations of the reactants. Specific glycosides of maltodextrins of varying degrees of polymerization (d.p.) are difficult and tedious to synthesize chemically, but with specific enzymes, such as CGTase, the synthesis is relatively facile.

In the present study, we report on the optimization of the *B. macerans* CGTase synthesis of specific sizes of maltodextrin glycosides, containing methyl α and β and phenyl α and β aglycones by using different molar ratios of 0.5–5.0 of the α and β anomers of methyl and phenyl D-glucopyranosides to cyclomaltohexaose (CD6). These reactions are compared with the syntheses of maltodextrins obtained when D-glucose was the acceptor.

2. Experimental

2.1. Materials

B. macerans CGTase was obtained by growing *B. macerans* ATCC 8517 on wheat bran medium and purified by a modification of the method of Kobayashi et al.,[15] as previously described.[16] Barley beta-amylase [EC 3.2.1.2] was purchased from Megazyme International (Wicklow, Ireland). CD6 and CD7 were obtained from Ensuiko Sugar Co. (Yokohama, Japan) and were pure by TLC analysis. Methyl α-D-glucopyranoside was purchased from Eastman Kodak Co. (Rochester, NY) and recrystallized twice in distilled water. Methyl β-D-glucopyranoside, phenyl α-D-glucopyranoside and phenyl β-D-glucopyranoside were obtained from Sigma Chemical Co. (St. Louis, MO) and found to be pure by TLC. Other chemicals were of reagent grade.

2.2. Assay of the activity of CGTase

CGTase activity was measured by a modification of the method of Thoma et al.[17] as previously described.[16] The substrate solution (1,490 μL), containing 18 mM CD6 and 18 mM methyl α-D-glucopyranoside in 25 mM imidazolium–HCl buffer (pH 6.0) was preincubated at 37 °C for 10 min. The enzyme reaction was started by adding 10 μL of CGTase to the substrate solution. Aliquots (150 μL) of the digest were taken every 5 min for 25 min and the reaction was stopped by heating in boiling water for 5 min. Glucoamylase (100 mU) was added to the heat treated CGTase digest and incubated at 37 °C for 20 min, and then heated in boiling water for 5 min to stop the reaction. The concentration of the glucose was determined by the micro glucose oxidase–peroxidase method.[18] One CGTase unit was defined as the number of μ moles of CD6 transferred to methyl α-D-glucopyranoside per min at the given conditions.

2.3. CGTase reaction conditions for the synthesis of maltodextrin glycosides

CGTase (200 mU) was added to 200 μL of the substrate solutions, containing 25 mM imidazolium–HCl buffer (pH 6.0), methyl α-D-glucopyranoside (or other glycosides) and CD6 in various molar ratios from 0.5 to 5.0. Two major concentrations of CD6 were used for the reactions with methyl α-D-glucopyranoside and D-glucose, 100 mM for molar ratios of 0.5–3.0 and 50 mM for molar ratios of 4.0 and 5.0; for the other glycosides, only 50 mM CD6 was used. The reactions were carried out at 37 °C and 30 μL aliquots were taken at 1, 3, 6, 9, 12, and 24 h. Equilibrium was reached in 12 h, but the reactions were allowed to go 24 h for analysis. The reactions were stopped by heating in a boiling water bath for 5 min, diluted 5-fold with water and analyzed by TLC. The synthesis of the maltodextrin glycosides was demonstrated by treating the digests with beta-amylase (10 U/mL) for 24 h at 37 °C and then analyzed by TLC.

2.4. Separation and quantitative determination of the CGTase coupling reaction products by TLC analysis

The CGTase coupling reaction products were separated by TLC, using the solvents and conditions given in Table 1. Each digest (1 μL) was spotted on a 20 × 20 cm Whatman silica gel TLC plate to give an 18 cm path length for 2 ascents of the solvents given in Table 1 for the different reactions. The carbohydrates were visualized by dipping the plate into a methanol solution, containing 0.3% (w/v) *N*-(1-naphthyl)ethylenediamine and 5% (v/v) H_2SO_4, dried and heated at 120 °C for 10 min. Maltodextrin standards and methyl α-D-glucopyranoside and cyclomaltodextrins (CD6, CD7, and CD8) were used as TLC standards to locate the products on the TLC plate. The maltodextrins were prepared by the reaction of *B. licheniformis* alpha-amylase (EC

Table 1. TLC conditions for the separation of the products from the methyl α, β and phenyl α, β D-glucopyranoside acceptor reactions with CD6 catalyzed by *B. macerans* CGTase

Reaction substrates	Solvent[a] volume proportions	Types of TLC plates	No. of 18 cm path length ascents
Me-α-Glc + CD6	85:25:55:50	Whatman K5	2
Me-β-Glc + CD6	85:20:50:50	Whatman K6	2
Ph-α-Glc + CD6	85:20:50:55	Whatman K5	2
Ph-β-Glc + CD6	85:25:55:50	Whatman K5	2

[a] Solvent = MeCN/EtOAc/1-PrOH/H_2O.

3.2.1.1) [Thermyl, Novo Industries, Copenhagen] with maize starch followed by reaction with isoamylase (EC 3.2.1.68) [Sigma Chemical Co., St. Louis, MO]. The addition of methyl and phenyl groups to D-glucose and maltodextrins increases the TLC mobilities of the glycosides with respect to the non-glycosides as shown in Figure 1. The identities of the maltodextrins and the maltodextrin glycosides were determined by comparing their mobilities with D-glucose and methyl α-D-glucopyranoside, respectively. The components and products of the enzyme reaction digests were quantitatively determined by scanning densitometry of the visualized carbohydrates on the TLC plate.[19,20]

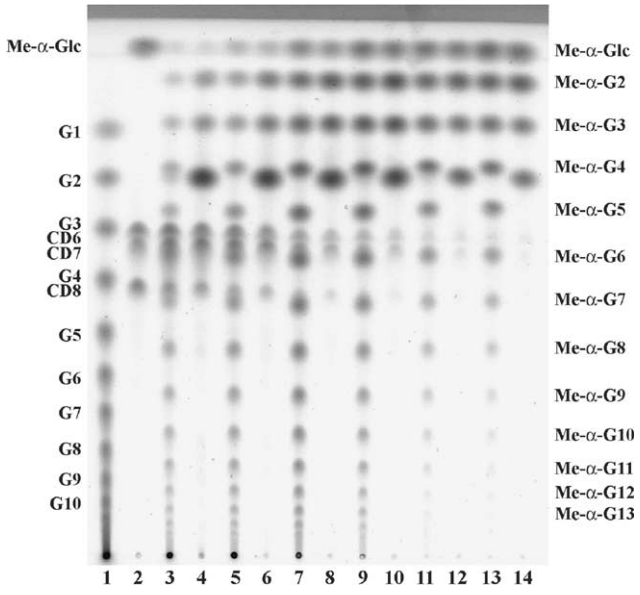

Figure 1. Thin layer chromatogram of CGTase reaction products and their reaction with beta-amylase. Lane 1, maltodextrin standards. Lane 2, cyclomaltodextrins (CD6, CD7, and CD8) and Me-α-Glc standards. Lane 3, reaction with 50 mM Me-α-Glc and 100 mM CD6 ($R = 0.5$). Lane 4, reaction of the products of lane 3 with beta-amylase. Lane 5, reaction with 100 mM Me-α-Glc and 100 mM CD6 ($R = 1.0$). Lane 6, reaction of the products of lane 5 with beta-amylase. Lane 7, reaction with 200 mM Me-α-Glc and 100 mM CD6 ($R = 2.0$). Lane 8, reaction of the products of lane 7 with beta-amylase. Lane 9, reaction with 300 mM Me-α-Glc and 100 mM CD6 ($R = 3.0$). Lane 10, reaction of the products of lane 9 with beta-amylase. Lane 11, reaction of 200 mM Me-α-Glc and 50 mM CD6 ($R = 4.0$). Lane 12, reaction of the products of lane 11 with beta-amylase. Lane 13, reaction of 250 mM Me-α-Glc and 50 mM CD6 ($R = 5.0$). Lane 14, reaction of the products of lane 13 with beta-amylase.

The densitometric method gives a linear and structurally independent response for D-glucose and each of the maltodextrins and maltodextrin glycosides. It allows the determination of the relative weight percent (or relative mole percent) of the individual maltodextrin or saccharide in a mixture by dividing the individual densities by the sum of the densities, and permits a quantitative determination without the use of an absolute standard.[19,20] The use of the relative weight percent or mole percent is important, in that it reduces the error in the analyses by eliminating the necessity of putting exactly equal amounts of carbohydrate on each TLC spot. This is particularly important in making comparisons between each individual reaction digest, as is done in this study.

3. Results and discussion

3.1. CGTase synthesis of methyl α-maltodextrin glycosides

The products of the reactions of CGTase with different molar ratios of methyl α-D-glucopyranoside to CD6 are presented in Table 2 and Figure 1. At relatively low molar ratios, $R = 0.5$ and 1.0, there was a significant amount of CD7 and CD8 formed and relatively equal molar amounts of Me-α-G2 to Me-α-G12 and appreciable amount of higher Me α-maltodextrin glycoside, d.p. 13 and higher. As the molar ratio was increased the higher maltodextrin glycosides decreased. The intermediate-sized maltodextrin glycosides, d.p. 9–4, reached a maximum at a ratio of 2.0; thereafter, they decreased and Me-α-G2 and Me-α-G3 reached a maximum at a ratio of 4.0. CD7 and CD8 also continued to decrease after a ratio of 1.0 and completely disappeared at a ratio of 3.0 (see Table 1). A ratio of 5.0 gave a decrease in all of the maltodextrin glycosides.

The CGTase reactions with Me-α-Glc and CD6 should not have given the formation of any maltodextrins. This was confirmed by reacting the CGTase digests with beta-amylase, which gave maltose, Me-α-Glc to Me-α-G3; no D-glucose or maltotriose was produced, both compounds that should have been formed if maltodextrins had been synthesized (see the even numbered lanes in Fig. 1).

3.2. CGTase synthesis of methyl β-maltodextrin glycosides

TLC analysis showed that a series of methyl β-maltodextrin glycosides were formed. The results of the reaction of CGTase with different ratios of methyl

Table 2. Percent molar composition of the products from the CGTase reactions with methyl α-D-glucopyranoside and cyclomaltohexaose in various molar ratios

CD6 (mM)[a]	100[c]	100	100	100	100	50[c]	50
Me-α-Glc (mM)	50	70	100	200	300	200	250
Molar ratio (R)[b]	0.5	0.7	1.0	2.0	3.0	4.0	5.0
CD6[a]	13.65	10.79	7.06	2.74	1.53	1.20	0.55
CD7[a]	9.87	8.03	6.03	0.66	—	—	—
CD8[a]	5.31	4.34	2.43	1.41	—	—	—
Mole % CDs	28.83	23.16	15.53	4.81	1.53	1.20	0.55
Me-α-Glc	9.75	10.85	14.55	23.45	35.17	37.48	45.50
Me-α-G2	7.74	9.55	12.84	16.50	21.17	23.15	22.75
Me-α-G3	4.10	7.54	9.61	12.04	12.85	14.19	12.82
Me-α-G4	4.10	6.45	7.40	9.02	8.83	8.51	8.26
Me-α-G5	5.01	6.83	7.21	8.15	6.95	5.86	4.86
Me-α-G6	6.32	6.11	6.10	6.39	4.81	3.88	2.61
Me-α-G7	4.32	3.81	3.69	4.27	3.07	2.19	1.28
Me-α-G8	2.82	3.65	3.49	2.80	1.85	1.21	0.64
Me-α-G9	2.04	2.63	2.47	2.18	0.96	0.67	0.27
Me-α-G10	2.60	2.40	2.28	2.14	0.99	0.67	0.26
Me-α-G11	2.26	2.15	2.02	1.62	0.75	0.49	0.13
Me-α-G12	1.60	1.43	1.33	1.29	0.53	0.34	0.06
Me-α-G13	0.41	0.38	0.33	0.43	0.12	0.07	0.01
Higher Me-α-Gn	18.12	13.07	11.17	4.90	0.42	0.10	0.01
Mole % of Me-α-Gn	61.42	65.99	69.92	71.74	63.3	61.32	53.95

[a] CD6, CD7, and CD8 represent cyclomaltohexaose, cyclomaltoheptaose, and cyclomaltooctaose, respectively; Gn = n number of D-glucopyranoside units.
[b] Molar ratio R = [Me-α-Glc]/[CD6].
[c] 1.0 unit/mL was used for 100 mM CD6 digests and 0.5 unit/mL was used for 50 mM CD6 digests.

β-D-glucopyranoside to CD6 are given in Table 3. A maximum yield of 68.2 mol % of the maltodextrin glycosides was obtained when the molar ratio was 1.0. As the molar ratio was increased to 2.0, there was a significant

Table 3. Percent molar composition of the products from the CGTase reaction with methyl β-D-glucopyranoside and cyclomaltohexaose in various molar ratios

CD6 (mM)[a]	100[c]	100	100	100	50[c]	50
Me-β-Glc (mM)	50	100	200	300	200	250
Molar ratio (R)[b]	0.5	1.0	2.0	3.0	4.0	5.0
CD6[a]	9.64	3.45	0.59	0.15	0.10	0.04
CD7[a]	13.43	6.17	1.81	0.62	0.22	0.08
CD8[a]	1.89	0.84	0.14	0.04	—	—
Mole % CDs	24.97	10.46	2.54	1.81	0.32	0.12
Me-β-Glc	11.10	21.30	34.99	45.67	55.59	57.47
Me-β-G2	11.19	16.38	23.06	23.12	21.49	21.22
Me-β-G3	5.24	8.73	11.21	9.41	7.61	7.70
Me-β-G4	4.76	8.51	7.56	6.31	5.39	5.32
Me-β-G5	5.50	6.81	6.01	4.95	3.78	3.50
Me-β-G6	3.70	2.91	2.30	2.04	1.67	1.64
Me-β-G7	3.33	3.24	2.83	2.18	1.58	1.39
Me-β-G8	2.61	2.99	1.88	1.27	0.70	0.53
Me-β-G9	2.37	2.41	1.39	0.94	0.46	0.31
Me-β-G10	1.67	1.79	1.03	0.64	0.31	0.18
Me-β-G11	3.19	2.13	1.22	0.83	0.40	0.26
Me-β-G12	3.57	2.12	1.09	0.60	0.32	0.17
Me-β-G13	3.16	1.84	0.97	0.53	0.23	0.11
Higher Me-β-Gs	13.63	8.38	1.94	0.69	0.18	0.09
Mole % Me-β-Gns	63.93	68.24	62.47	53.52	44.09	42.41

[a] CD6, CD7, and CD8 represent cyclomaltohexaose, cyclomaltoheptaose, and cyclomaltooctaose, respectively; Gn = n number of D-glucopyranoside units.
[b] Molar ratio R = [Me-β-Glc]/[CD6].
[c] 1.0 unit/mL was used for 100 mM CD6 digests and 0.5 unit/mL was used for 50 mM CD6 digests.

decrease in the maltodextrin glycosides, d.p. 4 and higher, although d.p. 4 and 5 were still significant, with mole percents of 7.6 and 6.0, respectively. Me-β-G2 and Me-β-G3 reached the maximum mole percents of 23.1 and 11.2, respectively, when the molar ratios were 2.0.

3.3. CGTase synthesis of phenyl α-maltodextrin glycosides

The results of the reaction of CGTase with different ratios of phenyl α-glucopyranoside to CD6 are given in Table 4. Higher d.p. maltodextrin glycosides of the phenyl series were separated by TLC up to d.p. 17. A molar ratio of 0.5 gave the largest amount of the higher d.p. products, although there was also a significant amount (18.1%) of CD7 formed. The largest overall yield (71.4%) of the maltodextrin glycosides was obtained when the molar ratio was 1.0 and there were significant amounts of d.p. 2–7 and 9 that were formed. With a molar ratio of 2.0, there were significant amounts of d.p. 2–5 that were formed. As the molar ratio was increased further, these same phenyl α-maltodextrin glycosides were formed, with increased amounts of d.p. 2–4. At a molar ratio of 5.0, the maximum amount (29.7%) of Ph-α-G2 was formed, with 16.4% Ph-α-G3 and 10.9% Ph-α-G4 and very low amounts of CDs.

3.4. CGTase synthesis of phenyl β-maltodextrin glycosides

The results of the reaction of CGTase with different ratios of phenyl β-D-glucopyranoside to CD6 are given in Table 5. As with the previous syntheses, the largest yields of the higher d.p. phenyl β-maltodextrin glycosides were obtained at the lower molar ratios of 0.5. The largest amounts (78.6%) of the phenyl β-maltodextrin glycosides were obtained with a molar ratio of 3.0, giving significant amount of Ph-β-G2 to Ph-β-G5, with Ph-β-G2 having a maximum amount of 34.1%. Relatively low amounts of CDs were formed with a molar ratio of 3.0 and higher.

3.5. CGTase synthesis of maltodextrins

The results of the reactions of CGTase with different molar ratios of D-glucose and CD6 to give the synthesis of maltodextrins are given in Table 6. The highest number of different sized maltodextrins, as well as the highest

Table 4. Percent molar composition of the products from the CGTase reaction with phenyl α-D-glucopyranoside and cyclomaltohexaose in various molar ratios

CD6 (mM)[a]	50[c]	50	50	50	50	50
Ph-α-Glc (mM)	25	25	25	25	25	25
Molar ratio (*R*)[b]	0.5	0.5	0.5	0.5	0.5	0.5
CD6[a]	7.21	2.29	1.55	0.73	0.62	0.20
CD7[a]	18.06	8.83	3.65	1.89	0.44	0.21
CD8[a]	2.23	0.66	0.17	0.05	0.03	0.01
Mole % CDs	27.50	11.78	5.37	2.67	1.09	0.42
Ph-α-Glc	13.95	16.85	26.27	27.27	34.78	35.90
Ph-α-G2	9.13	16.17	22.83	24.45	27.49	29.69
Ph-α-G3	12.97	18.07	16.73	19.08	16.60	16.37
Ph-α-G4	9.56	15.41	12.65	13.54	11.41	10.86
Ph-α-G5	7.15	9.76	8.87	8.61	5.98	4.94
Ph-α-G6	2.43	2.85	2.19	1.79	1.14	0.89
Ph-α-G7	1.95	1.83	1.55	1.08	0.70	0.47
Ph-α-G8	0.46	0.60	0.40	0.50	0.13	0.15
Ph-α-G9	2.46	2.23	0.95	0.49	0.29	0.18
Ph-α-G10	1.42	0.86	0.51	0.24	0.17	0.07
Ph-α-G11	1.48	0.81	0.39	0.18	0.10	0.03
Ph-α-G12	0.94	0.51	0.24	0.08	0.06	0.02
Ph-α-G13	0.74	0.30	0.13	0.03	0.03	—
Ph-α-G14	1.13	0.47	0.19	0.02	0.04	—
Ph-α-G15	1.11	0.36	0.11	—	—	—
Ph-α-G16	1.05	0.32	0.09	—	—	—
Ph-α-G17	0.34	0.08	0.02	—	—	—
Higher Ph-α-Gs	4.20	0.64	0.11	—	—	—
Total Mole % Ph-α-Gns[a]	58.55	71.37	68.36	70.09	64.13	63.69

[a] CD6, CD7, and CD8 represent cyclomaltohexaose, cyclomaltoheptaose, and cyclomaltooctaose, respectively; Ph-α-Gns = phenyl α-maltodextrin glycosides, containing *n* number of D-glucose units.
[b] Molar ratio = [Ph-α-Glc]/[CD6].
[c] 0.5 U/mL of CGTase was added for 50 mM CD6 digests.

Table 5. Percent molar composition of the products from the CGTase reaction with phenyl β-D-glucopyranoside and cyclomaltohexaose in various molar ratios

CD6 (mM)[a]	50[c]	50	50	50	50	50
Ph-β-Glc (mM)	25	50	100	150	200	250
Molar ratio (*R*)[b]	0.5	1.0	2.0	3.0	4.0	5.0
CD6[a]	9.77	5.54	2.58	1.30	0.78	0.56
CD7[a]	12.82	9.72	4.25	2.05	1.30	0.77
CD8[a]	3.13	1.06	0.26	0.10	0.06	0.02
Mole % CDs	25.72	16.32	7.09	3.44	2.15	1.35
Ph-β-Glc	9.59	8.25	15.35	17.94	20.66	24.28
Phβ-G2	10.53	15.58	24.63	28.19	32.85	34.10
Ph-β-G3	10.58	18.14	18.89	20.48	19.43	18.98
Ph-β-G4	10.15	15.95	15.91	15.99	14.39	13.20
Ph-β-G5	8.82	11.19	9.66	9.10	7.21	5.88
Ph-β-G6	2.33	2.79	2.36	1.87	1.37	1.03
Ph-β-G7	1.97	1.81	1.65	1.07	0.75	0.54
Ph-β-G8	1.17	1.64	1.04	0.68	0.45	0.33
Ph-β-G9	2.25	1.65	0.77	0.42	0.26	0.16
Ph-β-G10	1.44	1.10	0.67	0.30	0.18	0.09
Ph-β-G11	1.39	0.90	0.48	0.23	0.12	0.04
Ph-β-G12	0.99	0.61	0.31	0.10	0.06	0.02
Ph-β-G13	1.52	0.79	0.36	0.08	0.04	—
Ph-β-G14	1.56	0.74	0.31	0.07	0.03	—
Ph-β-G15	1.15	0.62	0.19	0.01	0.02	—
Higher Ph-β-Gs	8.84	1.93	0.31	0.03	0.03	0.01
Mole % Ph-β-Gns	64.69	75.43	77.56	78.62	77.19	74.37

[a] CD6, CD7, and CD8 represent cyclomaltohexaose, cyclomaltoheptaose, and cyclomaltooctaose, respectively; Ph-β-Gns = phenyl β-maltodextrin glycosides, containing *n* number of glucose units.
[b] Molar ratio = [Ph-β-Glc]/[CD6].
[c] 0.5 U/mL of CGTase was added for 50 mM CD6 digests.

Table 6. Percent molar composition of the products from the CGTase reactions with D-glucose and cyclomaltohexaose in various molar ratios

CD6 (mM)[a]	100	100	100	100	50	50
D-Glc (mM)	50	100	200	300	200	250
Molar ratio (*R*)[b]	0.5	1.0	2.0	3.0	4.0	5.0
CD6[a]	5.63	4.64	1.76	0.89	0.24	0.03
CD7[a]	7.97	6.10	2.03	1.04	0.36	0.05
CD8[a]–CD13	6.55	4.92	1.37	0.63	0.10	0.01
Total mole % CDs	20.15	15.66	5.16	2.56	0.70	0.90
D-Glc	8.44	16.97	32.49	41.77	60.18	64.00
G2	13.13	14.89	18.94	19.12	18.36	17.33
G3	7.50	8.07	10.40	11.93	10.40	8.74
G4	10.60	10.96	8.98	7.74	5.02	4.80
G5	10.22	9.44	7.46	5.87	3.37	3.12
G6	6.28	5.79	4.89	3.56	1.59	1.74
G7	6.10	5.62	4.23	2.56	0.16	0.12
G8	4.69	3.38	2.40	1.46	0.08	0.05
G9	2.63	2.24	1.64	1.00	0.04	0.02
G10	1.69	1.39	0.68	0.49	0.02	—
G11	0.33	0.30	0.20	0.18	—	—
G12 & higher	8.25	5.29	2.54	1.75	0.07	—
Total mole % of MDs	71.42	67.38	62.35	55.67	39.12	35.91

[a] CD6, CD7, and CD8 = cyclomaltohexaose, cyclomaltoheptaose, and cyclomaltooctaose, respectively; G2, G3, G4 = maltose, maltotriose, maltotetraose, and so forth, respectively; MDs = maltodextrins.
[b] Molar ratio, *R* = [D-Glc/CD6].
[c] 1.0 U/mL CGTase was used for 100 mM CD6 digests and 0.5 U/mL was used for 50 mM CD6 digests.

yield of 71.4% was obtained with a molar ratio of 0.5. This is in contrast to the syntheses of the four maltodextrin glycosides, where the highest number of different sized maltodextrins was also obtained at molar ratios

of 0.5, but the highest yields of the maltodextrin glycosides were obtained at the higher molar ratios: methyl α-maltodextrin glycosides at $R = 2.0$; methyl β-maltodextrin glycosides at $R = 1.0$; phenyl α-maltodextrin glycosides at $R = 3.0$; and phenyl β-maltodextrin glycosides at $R = 4.0$. As the molar ratio of D-glucose to CD6 was increased, the amounts of the higher d.p. maltodextrins were decreased, as they were for the maltodextrin glycosides but the overall yields of the maltodextrins were decreased. At a molar ratio of 3.0, the highest amounts of d.p. 2–8 were obtained. As the molar ratios were increased to 4.0 and 5.0, the amounts of the maltodextrins were further decreased.

3.6. Concluding discussion

The mechanism for the coupling reactions catalyzed by CGTase (see Fig. 2) involves first the binding of CD6 at the active site with the opening of the ring by the enzyme and the formation of a covalent, enzyme intermediate, between maltohexaose and the enzyme.[16] The acceptors, glucopyranoside glycosides in the present

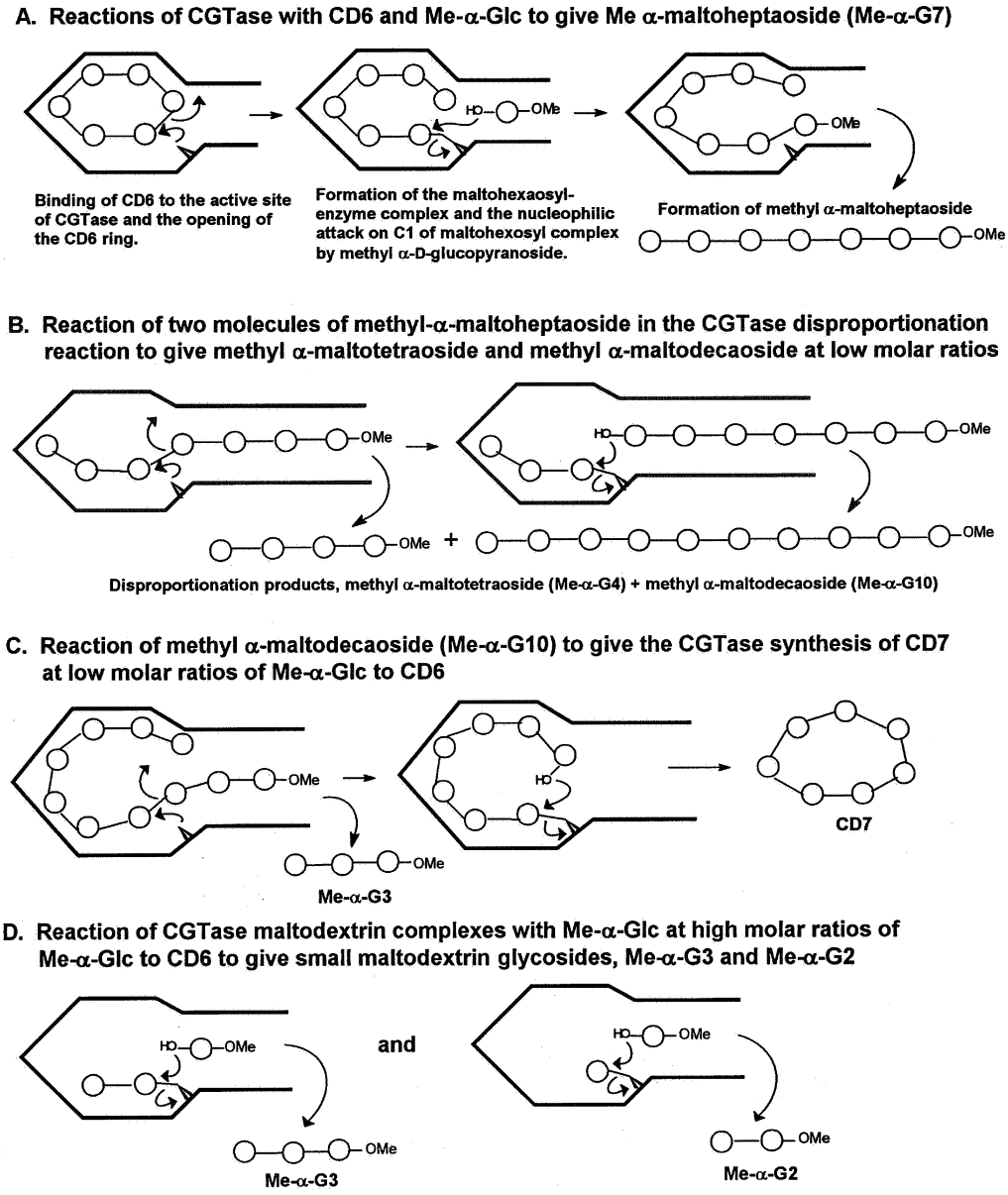

Figure 2. Reaction steps in the *B. macerans* CGTase coupling (acceptor) reactions. A. Opening of the CD6 ring and the formation of a covalent maltohexaosyl enzyme complex that reacts with Me-α-Glc acceptor to give Me-α-G7. B. Reaction of two molecules of Me-α-G7 with CGTase to give the disproportionation reaction at low molar ratios of Me-α-Glc to CD6. C. Reaction of methyl α-maltodecaoside (Me-α-G10) to give the CGTase synthesis of CD7 at low molar ratios of Me-α-Glc to CD6. D. Reaction of CGTase covalent maltodextrin–enzyme complexes with Me-α-Glc at high molar ratios of Me-α-Glc to CD6 to give small maltodextrin glycosides, Me-α-G3 and Me-α-G2.

study, then bind adjacent to the covalent, enzyme–maltohexaosyl unit, so that the C-4-OH of the glucoside makes a nucleophilic, S_N2 attack onto C-1 of the maltohexanoyl unit, giving an α-(1→4) linkage and the formation of a maltoheptaose glycoside. This first product then undergoes a CGTase catalyzed disproportionation reaction between two maltoheptaose glycoside molecules to eventually give a series of large and small maltodextrin glycosides.[1,4,5] At low molar ratios, the large maltodextrin glycosides are substrates for the formation of the higher cyclomaltodextrins, CD7 and CD8. At higher molar ratios, the large maltodextrin glycosides decrease and hence the formation of the cyclomaltodextrins also decreases. It should be noted that for *B. macerans* CGTase, CD6 is the preferred donor in the coupling reactions, and CD7 and CD8 are very poor donors.[7]

The formation of the glycosides of the maltodextrins gives a more stable material than maltodextrins in that the most reactive group in the maltodextrins is the hemiacetal, reducing end that can undergo a variety of reactions, such as oxidation, reduction, isomerization, derivatization, and so forth. The formation of the glycosides fixes the hemiacetal into an acetal with a specific α or β configuration that cannot undergo anomerization or other reactions. In different applications, the α or β anomers could each have advantages. The α anomer is more reactive than the β anomer in some kinds of reactions. For example, the α anomer is more easily hydrolyzed, whereas the C-2 position of the β anomer is more reactive than the C-2 position of the α anomer. Some enzymes can hydrolyze the α anomer but not the β anomer, and vice versa. Likewise, the methyl and phenyl aglycone groups could each have their own advantages, with the methyl group being a small alkyl group and the phenyl group being a much larger aromatic ring. Pure individual maltodextrin glycosides can be obtained by charcoal–Celite column chromatography on a relatively large, preparative scale.[21] Columns as large as 15 cm × 1.5 m, have successfully been used.

The selection of the proper molar ratios of the reactants in the CGTase synthesis can also afford mixtures with the enhancement of specifically sized maltodextrin glycosides. By using molar ratios of 0.5 and lower, a relatively large number of maltodextrins and maltodextrin glycosides can be obtained, especially those with d.p. values >10; by using ratios of 1.0–2.0, intermediate sized maltodextrin and maltodextrin glycosides with d.p. values 6–9 can be obtained; and by using molar ratios of 4.0–5.0, an enhancement of the smaller sized maltodextrin and maltodextrin glycosides with d.p. values of 2 and 3 can be obtained. Further, molar ratios of 0.5–1.0 gave significant, approximately equal mole percents of maltodextrin glycosides of d.p. 2–10, for all four types of the maltodextrin glycosides presented in this study, along with significant amounts of the higher d.p. maltodextrin glycosides with d.p.s 12 and higher.

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