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1 **Physicochemical functionality of chimeric isomaltomegalosaccharides with α -(1→4)-glucosidic**
2 **segments of various lengths**

3

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13

14 *Abbreviations:* DDase, dextran dextrinase; DP, average value of degree of polymerization; *Gn*,
15 maltodextrin with DP = *n*; IMS, isomaltomegalosaccharide; IMS-*p/q* (e.g., IMS-15/9), IMS with DPs
16 of *p* and *q* for α -(1→6)- and α -(1→4)-segments, respectively; *K_c*, stability constant; *K_s*, equilibrium
17 constant; MWCO-2000 and MWCO-3500, dialysis tubes with molecular weight cutoffs of 2,000 and
18 3,500, respectively; QC, quercetin; Q3G, quercetin-3-*O*- β -glucoside; TDT; thermal decomposition
19 temperature; TGA, thermogravimetric analysis; TNS, 2-*p*-toluidinylnaphthalene-6-sulfonate.

20

21 **Abstract**

22 Isomaltomegalosaccharide (IMS) is a long chimeric glucosaccharide composed of α -(1→6)-
23 and α -(1→4)-linked segments at nonreducing and reducing ends, respectively; the hydrophilicity and
24 hydrophobicity of these segments are expected to lead to bifunctionality. We enzymatically
25 synthesized IMS with average degrees of polymerization (DPs) of 15.8, 19.3, and 23.5, where α -
26 (1→4)-segments had DPs of 3, 6, and 9, respectively. IMS exhibited considerably higher water
27 solubility than maltodextrin because of the α -(1→6)-segment and an identical resistance to thermal

28 degradation as short dextran. Interaction of IMS with a fluorescent probe of 2-*p*-
29 toluidinylnaphthalene-6-sulfonate demonstrated that IMS was more hydrophobic than maltodextrin,
30 where the degree of hydrophobicity increased as DP of α -(1→4)-segment increased ($9 > 6 > 3$).
31 Fluorescent pyrene-estimating polarity of IMS was found to be similar to that of methanol or 1-
32 butanol. The bifunctional IMS enhanced the water solubility of quercetin-3-*O*-glucoside and quercetin:
33 the solubilization of less-soluble bioactive substances is beneficial in carbohydrate industry.

34

35 **Keywords:** dextran dextrinase, isomaltomegalosaccharide, chimeric structure, bifunctionality,
36 quercetin flavonoid, water solubility enhancement

37

38 1. Introduction

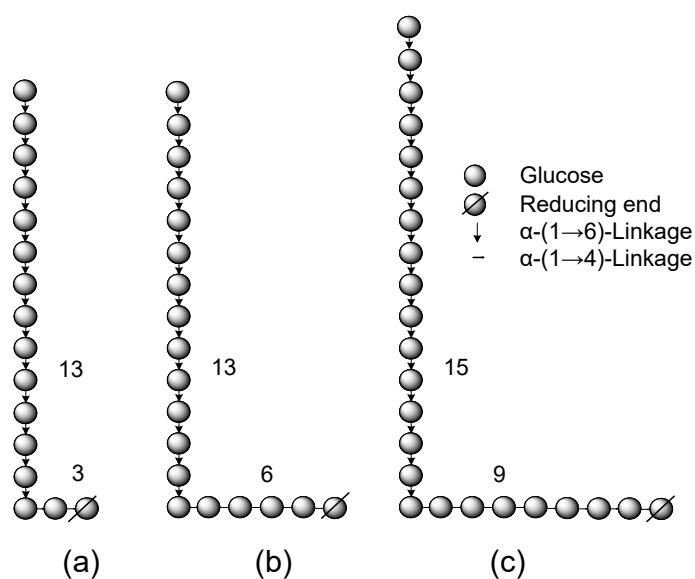
39 Glucosaccharides, such as soluble starch and dextran, are hydrophilic due to the presence of
40 numerous surface hydroxyl groups that promote water solubility. Monosaccharide glucose containing
41 methine groups has a negligibly weak hydrophobicity. However, α -(1→4)-glucosaccharides (i.e.,
42 maltodextrin and cyclodextrin) exhibit high hydrophobicity arising from stereochemical constrained
43 chains with helical structures (Balasubramanian et al., 1993), whereby the internal surface is relatively
44 apolar because all the methine groups point inwards. The degree of apolarity of the sugar chain can be
45 evaluated by hydrophobic interaction with fluorophore probe 2-*p*-toluidinylnaphthalene-6-sulfonate
46 (TNS) (Das et al., 1995) because the emission intensity of TNS is higher in a nonpolar state (i.e., in
47 sugar chain) than in an aqueous state. TNS interacts with the helical chain of maltodextrin where this
48 α -(1→4)-glucosaccharide forms a helical conformation for degrees of polymerization (DPs) greater
49 than 5 and an incipient helix-type structure for lower DPs (Sundari et al., 1991; Aoyama et al., 1992).
50 By contrast, dextran composed of sole α -(1→6)-glucosyl units possesses no hydrophobicity since this
51 α -(1→6)-glucosaccharide does not affect the fluorescence of TNS (Sundari & Balasubramanian, 1997),
52 indicating that dextran is a hydrophilic saccharide. Functional properties are affected by the type and
53 length of glucosaccharide chains. In this study, we hypothesize that a glucosaccharide containing both
54 α -(1→4)- and α -(1→6)-glucosidic segments ["chimeric" saccharide; i.e., isomaltomegalosaccharide

55 (IMS)] (Lang et al., 2022) could exhibit the bifunctionality, that is, both hydrophobicity and
56 hydrophilicity.

57 Dextran dextrinase (DDase; EC 2.4.1.2) is a glucosyltransferase that catalyzes a two-substrate
58 reaction with maltodextrins as the substrates. DDase can cleave an α -(1→4)-linkage at the
59 nonreducing end of a donor substrate and transfer the glucosyl unit to the nonreducing end of an
60 acceptor substrate to generate an α -(1→6)- or α -(1→4)-linkage (Yamamoto et al., 1992). Consecutive
61 transfers from the α -(1→4)-linkage result in the extension of the α -(1→6)-linked segment, whereby
62 the enzyme finally synthesizes a macromolecule of viscous dextran (Yamamoto et al., 1993).
63 Theoretically, the dextran produced by DDase is not an α -(1→6)-glucan because the reducing terminal
64 region is composed of α -(1→4)-linkages originating from maltodextrin of the acceptor substrate,
65 implying the product is a "chimeric" polysaccharide with α -(1→6)- and α -(1→4)-linked segments.
66 Recently, we found that intermediate-sized saccharides could be effectively produced by DDase
67 from *Gluconobacter oxydans* ATCC 11894 under optimized conditions (Lang et al., 2022). The
68 product is called an IMS based on the definition of a megalosaccharide (Thoma et al., 1959), for which
69 the DP ranges between that of an oligosaccharide (DP = 2–9) and a polysaccharide. The DP of a
70 polysaccharide is not well-defined, but the properties of polysaccharides emerge for DPs of more than
71 100 or 200 (Kitamura S, personal information); thus, the DP of a megalosaccharide is considered to be
72 10–100 (or 100–200). The IMS is a chimeric saccharide comprised of α -(1→6)- and α -(1→4)-
73 segments, where the α -(1→4)-segment is derived from a maltodextrin substrate. The IMS is expected
74 to possess a low viscosity and a high water solubility because the linear α -(1→6)-segment promotes
75 hydrophilicity, as suggested by Sundari et al. (1991) and Balasubramanian et al. (1993). An
76 *in vivo* study was performed to investigate the functionality of IMS. The IMS with short α -(1→4)-
77 segment enhanced the absorption of quercetin-3-*O*- β -glucoside (Q3G) in the rat small intestine
78 (Shinoki et al., 2013). In addition, higher enzymatic degradation of an azobenzene dye (ethyl red, an
79 environmental pollutant) by azoreductase occurred in the presence of an IMS than in the presence of
80 β -cyclodextrin possibly because of weak hydrophobic interaction between the IMS and ethyl red
81 (Lang et al., 2014). Strong interaction of β -cyclodextrin–ethyl red complex inhibited enzymic
82 degradation by its steric hindrance. Recently, an IMS was reported to enhance the barrier function of

83 intestinal epithelial tight junctions, which could facilitate inflammation suppression and reduce the
84 risks of chronic diseases (Hara et al., 2017).

85 In this study, we revealed the bifunctionality of the chimeric IMS with α -(1 \rightarrow 4)-segments of
86 various lengths: i.e., three IMSs with DPs of 15.8, 19.3, and 23.5 (IMS-13/3, IMS-13/6, and IMS-15/9,
87 respectively) (Fig. 1). Fluorescence studies were performed to determine the hydrophobic
88 characteristics of the IMS substrates: TNS was used to determine the stability constants (K_s), and the
89 sense of polarity was determined using polycyclic aromatic pyrene; to the best of our knowledge, the
90 sense of polarity was determined for the first time for carbohydrates in this study. The IMS-induced
91 water solubilization of Q3G and quercetin (QC) (which are bioactive flavonoids that are widely used
92 as health supplements) was also evaluated using a phase solubility diagram. The hydrophobicity of
93 IMS facilitates the aqueous solubilization of these flavonoids. Properties related to the α -(1 \rightarrow 6)-
94 segment (water solubility and thermal stability) were analyzed.



96 **Fig. 1.** Proposed structure of isomaltomegalosaccharide (IMS); a, IMS-13/3; b, IMS-13/6; c, IMS-15/9;
97 numbers, DPs (average values) of α -(1 \rightarrow 6)- and α -(1 \rightarrow 4)-segments.

98

99 2. Materials and methods

100 2.1. Materials

101 Q3G and QC hydrate were purchased from Extrasynthese (Cedex, France) and Tokyo

102 Chemical Industry (Tokyo, Japan), respectively. Dextran T1 (DP = 7), T1.5 (DP = 10), and T3.5 (DP

103 = 20) were obtained from Pharmacosmos (Holbaek, Denmark). Dextran T10 (DP = 60) and T40 (DP =
104 250) were purchased from Amersham Biosciences (Uppsala, Sweden). Amylose (DP = 28 and DP =
105 600; synthetic products) was purchased from Glico Nutrition (Osaka, Japan). Amylose (DP = 18; corn)
106 was purchased from Hayashibara (Okayama, Japan). TNS was obtained from Sigma–Aldrich
107 (Shinagawa, Japan). Pyrene, polar protic solvents, and polar aprotic solvents were purchased
108 from Nacalai Tesque (Kyoto, Japan). Cellulose dialysis membranes [with molecular weight cutoffs
109 of 2,000 (MWCO-2000) and 3,500 (MWCO-3500)] were purchased from Spectrum (Rancho
110 Dominguez, CA, USA). Maltotriose (G3) and G6/G7 (maltohexaose and maltoheptaose; commercial
111 product Fugioligo) were donated by Nihon Shokuhin Kako (Tokyo, Japan). Maltodextrin with average
112 DP = 11.8 (G12), of which DP ranged in 11.2–13.6 (see Fig. 2C), was prepared by the hydrolysis of
113 short amylose (DP = 16.5–19.5; Carbosynth, Berkshire, UK) as follows (Fig. S1): short amylose (100
114 mg/mL) was suspended in a 50 mM sodium acetate buffer (pH 5.0) containing 0.02% sodium azide
115 and treated with recombinant pullulanase (5 μ L/mL; Sigma–Aldrich, St. Louis, MO, USA) at 37 °C
116 for 2 d to cleave the α -(1→6)-linkage; the G12 fraction was then isolated by precipitation with 75%
117 (v/v) methanol and dried *in vacuo* (45% yield). The ¹H-NMR analysis of isolated G12 is shown in Fig.
118 S2A and S2B.

119

120 **2.2. Preparation of freeze-dried DDase**

121 Cultivation of *G. oxydans*, extracellular DDase preparation, and enzyme activity assays were
122 performed according to methods previously used (Lang et al., 2022). Suitable quantities of cells were
123 suspended in a 25 mM sodium acetate buffer (pH 4.2, 50 mL) containing 1% G3 and incubated at
124 30 °C for 3 h under agitation at 200 rpm (rev/min). During incubation of the cells with G3, DDase was
125 secreted extracellularly and produced dextran from G3, which was tightly bound to DDase (dextran-
126 bound DDase) (Lang et al., 2022). The resultant dextran-bound enzyme was dialyzed against water
127 containing 0.02% sodium azide at 4 °C for 2 d and then frozen at -80 °C. Dried DDase was obtained
128 by freeze-drying for 2 d using a lyophilizer FDU-1200 (Eyela, Tokyo, Japan).

129

130 **2.3. Production and structural analysis of IMS**

131 As previously reported (Lang et al., 2022), increasing the substrate concentration promoted
132 IMS formation and depressed dextran formation, and increasing the agitation efficiency increased the
133 α -(1 \rightarrow 6)-linkage content of IMS and decreased dextran formation. Freeze-dried DDase (0.1 U/mL)
134 was mixed with 200 mM G3, G4, G6/G7 or G12 containing a 50 mM sodium acetate buffer (pH 4.2),
135 where the reaction volume was 40 mL, and incubated at 50 °C for 96 h in a 200-mL baffled flask
136 under agitation at 100 rpm. An appreciable quantity of the mixture (100–200 μ L) was recovered at the
137 designated time. The reaction was terminated by heating at 100 °C for 20 min, followed by
138 centrifugation at 12,000 \times g for 10 min at 4 °C to remove the denatured enzyme. The progress of the
139 formation of the α -(1 \rightarrow 6)-linkage of saccharides contained in the reaction mixture was monitored
140 using 1 H-NMR according to a method we have previously reported (Lang et al., 2022).

141 The reaction was allowed to proceed for 96 h, after which IMS and dextran were separated by
142 methanol fractionation based on a procedure we have previously reported (Lang et al., 2022), except
143 that 50% (v/v) methanol was used to precipitate the dextran generated from G3 or G4. The IMSs
144 obtained from G6/G7 and G12 were further purified by dialysis with MWCO-2000 and MWCO-3500,
145 respectively, for 18 h at 4 °C. The IMSs were desalted using an ion exchange resin (Lang et al., 2014).
146 The IMS obtained from G6/G7 was treated with porcine pancreatic α -amylase (Sigma–Aldrich) to
147 shorten the length of the reducing terminal α -(1 \rightarrow 4)-segment (Lang et al., 2022). The carbohydrate
148 concentration was measured by the phenol–sulfuric acid method (Dubois et al., 1959) using a glucose
149 standard. Both the average value of DP and type of α -glucosyl linkage of the IMSs were analyzed by
150 1 H-NMR (Lang et al., 2014) (Fig. S2C and S2D). The saccharide size distribution was estimated using
151 gel permeation HPLC (Lang et al., 2022). According to our previous method (Lang et al., 2022), the
152 length of α -(1 \rightarrow 4)-chain of IMS was investigated by digestion with dextran glucosidase, which
153 catalyzes the exo-wise hydrolysis of α -(1 \rightarrow 6)-glucosidic linkage of substrate at nonreducing end and
154 no attack on α -(1 \rightarrow 4)-glucosidic linkage (Saburi et al., 2006), and the formed maltodextrins were
155 analyzed. Water content of IMS is shown in Table S1.

156

157 **2.4. Thermal properties and water solubility**

158 The thermal decomposition of the IMSs was measured with a thermogravimetric analysis
159 (TGA) apparatus (TG 8120; Rigaku, Tokyo, Japan). An aluminum crucible was used to hold 10 mg of
160 an oven-dried sample at 40 °C for 1 d. TGA was performed by elevating the temperature from 50 to
161 500 °C at a heating rate of 10 °C/min under an N₂ atmosphere with a flow rate of 50 mL/min. The data
162 were analyzed using the Thermo plus EVO version 2.060-1 software program (Rigaku).

163 Water solubility was determined by dissolving the IMS sample (40 mg) in 0.1 mL of water
164 using a 1.5-mL microcentrifuge tube. The liquid in the tube was mixed thoroughly and left to stand at
165 25 °C for 5 h. The suspension was centrifuged at 12,000× g at 25 °C for 20 min. The supernatant (50
166 μL) was lyophilized and weighed, and the results were used to calculate the dissolved quantity of the
167 sample in 1 mL of water. Commercial samples of three amyloses and four dextrans were analyzed in
168 the same way.

169

170 **2.5. TNS binding analysis**

171 Fluorescence titration was conducted to investigate the formation of complexes between 0–10
172 mM IMS and 10 μM TNS in water on a Hitachi F-4500 spectrometer (Tokyo, Japan) at an excitation
173 wavelength of 360 nm according to a previously reported method (Buranaboripan et al., 2014). K_c was
174 determined from Benesi–Hildebrand plots [Eq. (1)] at an emission intensity of 447 nm for IMS-13/3
175 and IMS-13/6 and 467 nm for IMS-15/9.

$$176 \quad 1/(I - I_0) = 1/(I' - I_0) + 1/\{K_c(I' - I_0)[H]\} \quad (1)$$

177 In the equation above, I_0 , I and I' are the initial fluorescence intensities of TNS without saccharide,
178 with saccharide of different concentrations ($[H]$), and with saccharide at the maximum concentration,
179 respectively.

180

181 **2.6. Polarity estimated by pyrene**

182 Ten μL pyrene in ethanol was added to saccharide in water (final volume, 1 mL), and then the
183 fluorescence spectra were immediately monitored at 25 °C on a Hitachi F-4500 spectrometer at an
184 excitation wavelength of 335 nm, where the ratio of the emission intensity at 375 nm (I_1) to that at 384
185 nm (I_3) was estimated. Final concentrations of pyrene, saccharide, and ethanol were 0.5 μM, 20–100

186 mM, and 1% (v/v), respectively. The I_1/I_3 value of 1% (v/v) ethanol solution was measured to be 1.4.
187 The same approach was applied to estimate the I_1/I_3 values of polar protic solvents (e.g., methanol)
188 and polar aprotic solvents (e.g., dimethyl sulfoxide). The determined I_1/I_3 values and dielectric
189 constants for the solvents (<https://macro.lsu.edu/howto/solvents/Dielectric%20Constant%20.htm>) were
190 used to generate calibration curves (see Fig. 3D) to evaluate the polarities of the IMSs and dextrans.

191

192 **2.7. Phase solubility of flavonoids**

193 An excess quantity of Q3G or QC (1.0 mg) was mixed with 100 μ L of an aqueous solution
194 containing 0–40 mM saccharide. The suspension was mixed frequently, left to stand at 25 °C for 6 h,
195 and a solution saturated with Q3G or QC was recovered using a previously reported approach (Lang et
196 al., 2014). A portion of the saturated solution was diluted with dimethyl sulfoxide and used to quantify
197 the flavonoid by measuring the absorbance at 360 nm. The apparent equilibrium constant (K_s) was
198 estimated from the phase solubility diagram (Higuchi and Connors, 1965) according to a method we
199 have previously reported (Lang et al., 2014).

200

201 **3. Results and discussion**

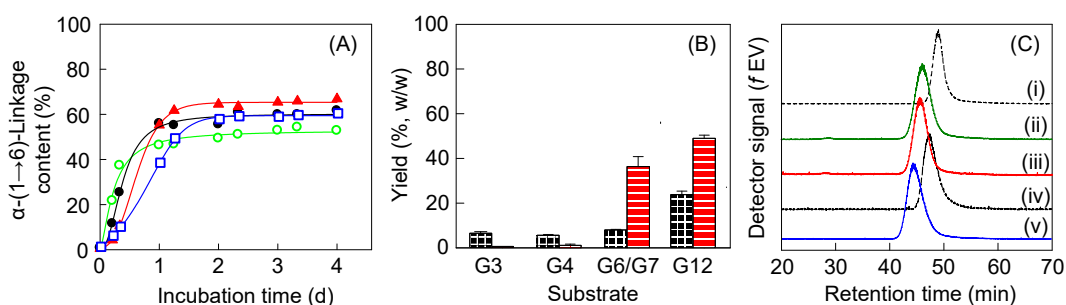
202 **3.1 Production of IMSs with α -(1→4)-segments of various lengths at the reducing end**

203 *G. oxydans* DDase, a cell surface protein, is secreted from cells when starch hydrolysate
204 (maltodextrin or glucose) is present in the medium (Suzuki et al., 1999; Sadahiro et al., 2015; Lang et
205 al., 2022). Cultivation of cells with maltodextrin (except G2) results in the extracellular generation of
206 dextran-bound DDase, which is more thermostable than the dextran-free enzyme (Lang et al., 2022). It
207 is also known that coexistence with dextran stabilizes the activities of freeze-dried enzymes (Allison et
208 al., 1998; Anchordoquy et al., 2001), suggesting that dextran-bound DDase maintains its activity after
209 freeze-drying and can be stored for long periods. Dextran-bound DDase (0.127 U/mL) was obtained
210 from the medium in which cells (corresponding to 31.5 mg dry cells) were treated with 1.0% G3. The
211 enzyme maintained 94% of its original activity after lyophilization, which makes extended storage of
212 dextran-bound DDase feasible.

213 A 200 mM substrate (G3, G4, G6/G7, or G12) was reacted with 0.1 U/mL freeze-dried DDase
 214 for 96 h according to our previous approach (Lang et al., 2022), except that the incubation temperature
 215 was increased from 45 °C to 50 °C, without affecting the IMS production yield. Incubation at 50 °C
 216 decreased the viscosity of the reaction mixture containing the highly concentrated (200 mM) substrate
 217 and encumbered the retrogradation of G12. Long maltodextrin G12 immediately thickened to form a
 218 gel at a lower temperature.

219 We recovered the reaction mixture at the indicated time, and all the saccharides contained in
 220 the reaction mixture (remaining substrate and formed products) were subjected to ¹H-NMR monitoring
 221 (Fig. 2A). The degree of formation of the α -(1→6)-linkage increased in a sigmoidal manner due to α -
 222 (1→4)-linkage formation by DDase (Lang et al., 2022). Table 1 shows that L_{max} [the maximum α -
 223 (1→6)-linkage content of all the saccharides] increased from 56.7 to 66.3% for G3–G6/G7 as the DP
 224 of the substrate increased. The L_{max} value of G12 (62.7%) is an exception of this manner since highly
 225 concentrated G12 (200 mM) gradually retrograded during the long reaction, even at 50 °C. The
 226 reaction time (T_{50}) at which half of L_{max} was obtained increased in the order G12 > G6/G7 > G4 > G3
 227 (Table 1), indicating that α -(1→6)-linkage formation was slower for a longer substrate.

228



229

230 **Fig. 2.** Production of isomaltomegalosaccharide (IMS). **A**, Increase in the α -(1→6)-linkage content of
 231 all saccharides in the reaction mixture. \circ , G3; \bullet , G4; \blacktriangle , G6/G7; \square , G12. **B**, Yield of dextran (black)
 232 and IMS (red) isolated from the reaction mixture for a 96-h reaction time. Yield (%; w/w), amount of
 233 each product per that of substrate used. **C**, Size distribution of IMS (ii, iii, or v) and substrate (i or iv).
 234 i, G6/G7; ii, IMS-13/3; iii, IMS-13/6; iv, G12; v, IMS-15/9.

235

236 **Table 1.** Formation of α -(1 \rightarrow 6)-linkages in saccharides in the reaction mixture and characterization of
 237 the isomaltomegalosaccharide (IMS) fraction.

200 mM Substrate	All saccharides in reaction mixture		Isolated IMS		
	L_{\max}^a	T_{50}^b	DP	α -(1 \rightarrow 6)-content	DP of two chains ^c
	(%)	(h)		(%)	
G3	56.7	5.9	NO	NO	NO
G4	61.1	9.8	13.2	70.3	4: 9
G6/G7	66.3	14.3	19.3	69.3	6: 13
G12	62.6	18.0	23.5	63.2	9: 15

238 ^a Maximal α -(1 \rightarrow 6)-linkage content of all saccharides.

239 ^b Reaction time at which half of L_{\max} was obtained.

240 ^c The first and second values denote the DPs of the α -(1 \rightarrow 4)- and α -(1 \rightarrow 6)-segments, respectively.

241 NO was not obtained because of a low IMS yield from G3.

242

243 The subsequent DDase reaction at 96 h showed that an adequate content of α -(1 \rightarrow 6)-segment
 244 was generated for each substrate (Fig. 2A); therefore, the product was separated into IMS and dextran
 245 by methanol fractionation. The IMSs from G6/G7 and G12 were subjected to further purification by
 246 dialysis using MWCO-2000 and MWCO-3500, respectively, to remove saccharide components with
 247 DPs below 12 and 22, respectively. Fig. 2B shows the yield of purified IMS or dextran (w/w, as a
 248 percentage of the initial quantity of substrate), demonstrating that the production of both IMS and
 249 dextran increases with the DP of the maltodextrin substrates. IMSs were obtained in a high yield from
 250 the reaction of G6/G7 and G12 (36 and 49%, respectively) but in a very low yield from the reaction of
 251 G3 and G4 (0.04 and 0.21%, respectively), where the main products were oligosaccharides. Even for a
 252 small yield, the IMS from G4 had a DP of 13.2, as analyzed by ¹H-NMR. When freeze-dried DDase
 253 was reacted with highly concentrated G3 (500 mM) for 96 h, the IMS yield increased to 15.8%, but
 254 the DP of the α -(1 \rightarrow 6)-segment was low [DP = 12.0 with nine α -(1 \rightarrow 6)-glucosyl units].

255 The IMSs produced from G6/G7 and G12 (corresponding to IMS-13/6 and IMS-15/9,
 256 respectively) had the estimated DPs of 19.3 and 23.5, respectively (Table 1), and contained α -(1 \rightarrow 4)-
 257 glucosyl units with average DPs of 6 and 9 at the reducing ends, respectively (Fig. 1). We used IMS-

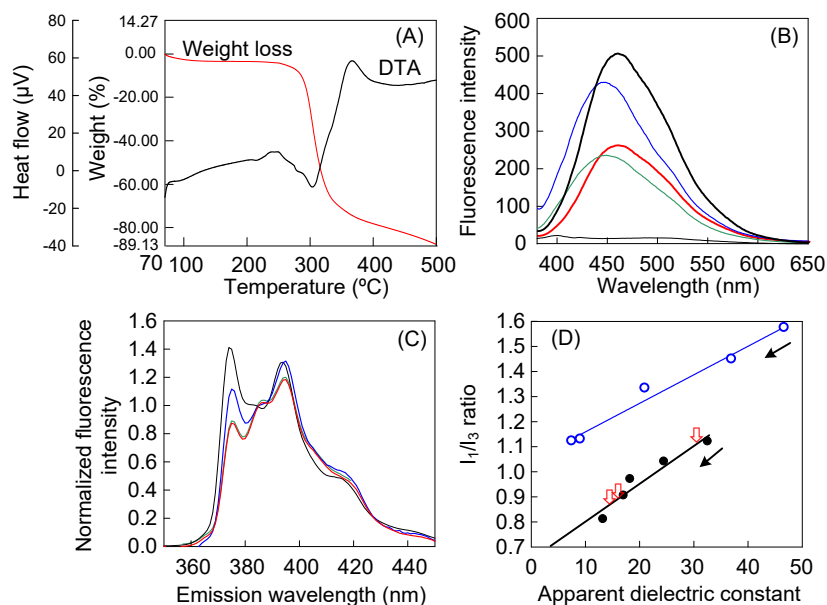
258 13/6 as a substrate, for which the α -(1→4)-segment was selectively cleaved by α -amylase (Lang et al.,
259 2020) and converted into a maltodextrin moiety (average DP = 3) to generate an additional IMS (IMS-
260 13/3) with DP = 15.8. The size distributions of the three IMSs were compared with that of the
261 substrate used (Fig. 2C). Chain length of α -(1→4)-segment of each IMS was analyzed by dextran
262 glucosidase digestion (Lang et al., 2020), and maltodextrins corresponding to α -(1→4)-segment were
263 formed (Fig. S3). The IMS structures are shown in Fig. 1, where IMS-13/3 and IMS-13/6 have the
264 same α -(1→6)-segment structure with average DP = 13, and IMS-15/9 has a longer α -(1→6)-segment
265 with average DP = 15. The α -(1→6)-segment contents were 83.3, 69.3, and 63.2% for IMS-13/3, IMS-
266 13/6, and IMS-15/9, respectively.

267

268 **3.2 Thermal decomposition and water solubility of IMS**

269 The activation energy for amylopectin is higher compared with amylose (Pigłowska et al.,
270 2020). This phenomenon can be explained by its high molecular weight and α -(1→6)-segment content
271 (Liu et al., 2010), suggesting that the α -(1→6)-linked saccharide is more stable than the α -(1→4)-
272 linked saccharide. Therefore, an IMS containing these two segments should be more stable than
273 amylose with a similar size. To investigate the IMS chimeric property, we used TGA to measure the
274 apparent thermal decomposition temperature (TDT) of the IMSs, with amylose and dextran as control
275 saccharides. Under heating in a TGA apparatus in an N₂ atmosphere, IMS-15/9 remained unchanged
276 below 260 °C except for moisture loss, followed by thermal decomposition corresponding to a total
277 weight loss of 89.13% at 500 °C (Fig. 3A). Similar curves were observed for the other samples. The
278 decomposition peak in the differential thermal analysis curve (Fig. 3A) was used to estimate the TDT of
279 each saccharide (Table 2). The TDT of amylose increased with DP, whereas that of dextran did not
280 [Table 2 shows that dextran T10 (DP = 60) and T40 (DP = 250) have lower TDTs than T1 (DP = 7)].
281 This result is probably due to the low quantities of other glucosidic linkages, such as α -(1→2)- or α -
282 (1→3)-bonds, in the chains of the dextrans T10 and T40 (commercially available samples). A
283 comparison of the TDTs of saccharides with nearly identical DPs [i.e., dextran T3.5 (DP = 20; TDT =
284 304.7 °C), IMS-13/6 (DP = 19.3; TDT = 304.7 °C), and amylose (DP = 20; TDT = 297.6 °C

285 (Saavedra-Leos et al., 2015)] shows that the thermal stability of IMS is similar to that of dextran and
 286 higher than that of amylose.



287
 288 **Fig. 3.** Characterization of isomaltomegalosaccharide (IMS). **A**, Thermal decomposition of IMS-15/9;
 289 DTA, differential thermal analysis. **B**, Emission spectra of 10 μM TNS in water (thin black line) and in
 290 10 mM glucosaccharide; green, IMS-13/3; blue, IMS-13/6; thick black, IMS-15/9; red, G7. **C**,
 291 Normalized pyrene emission spectra; black, water containing 1 % (v/v) ethanol (blank); blue, IMS-
 292 13/3; green, IMS-13/6; red, IMS-15/9. **D**, Calibration curves for I₁/I₃ versus the apparent dielectric
 293 constant: polar protic solvents (●, in the direction of the black arrow, methanol, ethanol, 2-propanol, 1-
 294 butanol, and 1-hexanol; $Y = 0.015X + 0.647$, $R^2 = 0.922$: Y and X represent I₁/I₃ and apparent
 295 dielectric constant, respectively) and polar aprotic solvents (○, in the direction of the black arrow,
 296 dimethyl sulfoxide, acetonitrile, acetone, dichloromethane, and tetrahydrofuran; $Y = 0.011X + 1.047$,
 297 $R^2 = 0.979$). Red arrows from left to right, IMS-15/9, IMS-13/6, and IMS-13/3 (100 mM for each).
 298 Ethanol solution of pyrene was used, and the final concentration of ethanol was 1% (v/v).

299 **Table 2.** Physical properties of isomaltomegalosaccharide (IMS), amylose, and dextran.

Glucosaccharide (DP)	Decomposition temperature (°C)	Water solubility at 25 °C (mg/mL)	K_c by TNS (M ⁻¹)	I_1/I_3 determined by pyrene ^a (dielectric constant ^b)			K_s for Q3G (M ⁻¹)	K_s for QC (M ⁻¹)
				100 mM	40 mM	20 mM		
IMS								
IMS-13/3 (15.8)	297.1	> 400	14.8	1.12 (31.3)	1.24 (39.5)	1.39 (49.5)	9.0	1.4
IMS-13/6 (19.3)	304.7	> 400	55.2	0.88 (15.8)	0.92 (18.2)	0.95 (20.2)	12.5	1.5
IMS-15/9 (23.5)	305.7	> 400	114	0.87 (15.1)	0.89 (16.2)	0.93 (18.9)	56.3	3.7
Amylose								
G5 (5)			1.9 ^c					
G6 (6)			8.8 ^c					
G7 (7)			27 ^c				6.01	0
Corn (18)		15.5 ± 2.1						
Amylose (20)	279.6 ^d							
Synthetic (28)	304.2	10.5 ± 1.5						
Synthetic (600)	309.8	0.52 ± 0.02						
Dextran								
T1 (7)	302.4	> 400		1.29 (42.9)	1.34 (46.2)	1.38 (48.9)	5.73	0
T1.5 (10)				1.25 (40.1)	1.25 (40.2)	1.29 (42.9)	5.86	0
T3.5 (20)	304.7	> 400		1.27 (41.2)	1.28 (42.2)	1.28 (42.2)		
T10 (60)	297.9	> 400		1.31 (44.5)	1.33 (45.5)	1.32 (44.9)		
T40 (250)	299.9	> 400 (swelling)						

300 ^a Pyrene in ethanol solution was added to saccharide sample, where the final concentration of ethanol was 1% (v/v).

301 ^b Estimated using the standard curve presented in Fig. 3D.

302 ^c Aoyama et al., 1992.

303 ^d Saavedra-Leos et al., 2015.

304 IMS possessed a high water solubility identical to that of short dextran (T3.5; DP = 20), i.e., >
305 400 mg/mL at 25 °C (Table 2). Large dextran (T40; DP = 250) maintained a high water solubility and
306 became swollen and very viscous. By contrast, amylose with DP = 18–28 exhibited a low solubility of
307 15–10 mg/mL (Table 2). Long amyloses typically have poor water solubility (Mukerjea & Robyt,
308 2010) because the crystalline packing of double helices in A- and B-type conformations reduces the
309 binding capacity of water (Naknean & Meenune, 2010). The results obtained indicate that the high
310 aqueous solubility of IMS derives from the α -(1→6)-segment. Hence, our findings elucidate the effect
311 of the α -(1→6)-segment on thermal stability and water solubility, indicating the advantage offered by
312 the chimeric IMS structure.

313

314 **3.3 Using fluorescent TNS and pyrene to monitor the hydrophobicity of IMS**

315 The hydrophobic interaction between TNS and dextran was reported as undetectable, because
316 the methine groups in a flexible twofold crankshaft-like conformation of dextran are turned toward the
317 bulk water medium (Sundari & Balasubramanian, 1997). However, the α -(1→4)-segment of amylose
318 considerably increases the degree of hydrophobicity, the extent of which depends on the chain length,
319 which generates curved nonpolar surfaces or several helical forms. The hydrophobic interaction is
320 weak between TNS and G3 and not very selective at binding, but it is detectable for maltodextrin with
321 DP of 5 or higher. Perhaps maltodextrins with DP \geq 5 can undergo an induced-fit adjustment to
322 interact with hydrophobic ligands (e.g., TNS), similar to the hydrophobic cavity of cyclodextrin
323 (Aoyama et al., 1992). The K_{cs} of G5 (maltopentaose), G6, and G7 have been estimated using TNS to
324 be 1.9, 8.8, and 27 M⁻¹, respectively (Aoyama et al., 1992). These results suggest that IMS possesses
325 hydrophobicity that mainly originates from the α -(1→4)-segment. The K_{cs} of IMS-13/3, IMS-13/6,
326 and IMS-15/9 were evaluated to be 14.8, 55.2, and 114 M⁻¹, respectively (Fig. S4), as expected (Table
327 2), and increase in the order of increasing length of the α -(1→4)-segment (average DP = 9 > 6 > 3).
328 The fluorescence intensity of IMS-15/9 was significantly increased, and the maximum wavelength
329 shifted to 467 nm from 447 nm for IMS-13/3 and IMS-13/6 (Fig. 3B), suggesting that the hydrophobic
330 area of IMS-15/9 occupies a more polar environment than of IMS-13/3 and IMS-13/6 (Barel, 1975).
331 The same phenomenon was also observed for G7, where the maximum wavelength was 467 nm (Fig.

332 3B), which further supports that the high hydrophobicity of IMS-15/9 originates from the α -(1→4)-
333 segment.

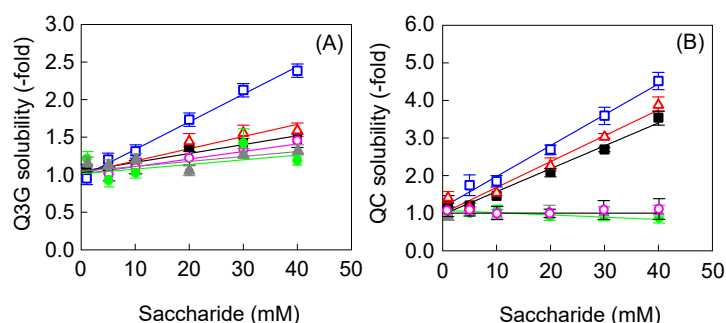
334 Furthermore, the polarity of the IMS hydrophobicity was examined by monitoring the
335 fluorescence spectrum of the complex formed between 0.5 μ M pyrene and 100 mM IMS (Fig. 3C),
336 which was used to estimate I_1/I_3 (Table 2). A high I_1/I_3 indicates a high polarity. The I_1/I_3 s of IMS-13/3,
337 IMS-13/6, and IMS-15/9 showed that the I_1/I_3 decreased as the length of the α -(1→4)-segment of IMS
338 increased, indicating that the IMS hydrophobicity derives from the α -(1→4)-segment. The α -(1→6)-
339 saccharide dextran (DP = 7–60; 100 mM) possessed larger I_1/I_3 values than 100 mM IMS (Table 2).
340 We measured I_1/I_3 for polar protic and polar aprotic solvents. $I_1/I_3 = 0.81–1.12$ was measured for polar
341 protic solvents, which contain a hydroxy group (and/or other polar protic groups) that can donate a
342 proton, whereas $I_1/I_3 = 1.12–1.57$ was measured for polar aprotic solvents that do not contain such
343 groups. The following solvents were investigated: polar protic solvents, methanol ($I_1/I_3 = 1.12$;
344 dielectric constant = 32.7), ethanol (1.04; 24.6), 2-propanol (0.97; 19.9), 1-butanol (0.91; 17.5), and 1-
345 hexanol (0.81; 13.3); polar aprotic solvents, dimethyl sulfoxide (1.57; 46.7), acetonitrile (1.45; 37.5),
346 acetone (1.33; 20.7), dichloromethane (1.13; 8.93), and tetrahydrofuran (1.12; 7.58). Fig. 3D shows
347 the calibration curves (I_1/I_3 versus the dielectric constant) of the polar protic and aprotic solvents, and
348 the calibration curves of the polar protic solvents was used to estimate the IMS dielectric constants
349 (Table 2), because carbohydrates have polar hydroxyl groups. These dielectric constants indicate that
350 the polarities of IMS-13/3, IMS-13/6, and IMS-15/9 are comparable to methanol, 1-butanol, and 1-
351 butanol, respectively. Table 2 shows that the I_1/I_3 s of 20 and 40 mM IMS are lower than those of the
352 20 and 40 mM dextrans, demonstrating that IMS hydrophobicity is maintained at low concentrations.
353 Fluorescence studies using TNS and pyrene indicate that the IMS hydrophobicity mainly originates
354 from the α -(1→4)-segment.

355

356 **3.4 Solubility enhancement of QC and Q3C by IMS**

357 QC flavonoids are known to have poor water solubility: $4.7 \pm 0.4 \mu$ M for QC and 156.3 ± 5.5
358 μ M for Q3G at 25 °C in water (both showed pH 6.80). This characteristic derives from the methyl
359 groups and *iso*-pentyl groups in these structures that increase lipophilicity (Crozier et al., 2009), which

360 results in poor solubility. β -Cyclodextrin or its derivatives have been successfully used to improve the
 361 solubility of these flavonoids by forming inclusion complexes (Sun et al., 2008; Pinho et al., 2014).
 362 We analyzed the IMS-mediated water solubility of QC flavonoids. A phase solubility diagram assay
 363 was employed for this purpose. The solubility curves of Q3G and QC in water were classified as the
 364 A_L type (Higuchi & Connors, 1965) for all the IMSs, indicating that the solubility increased with the
 365 IMS concentration (Fig. 4). The K_{sS} for QC and Q3G (Table 2) decreased in the order IMS-15/9 >
 366 IMS-13/6 > IMS-13/3, that is, as the length of the α -(1 \rightarrow 4)-segment decreased. G7 exhibited lower
 367 solubilization of both flavonoids than the IMSs (Fig. 4), probably because of the low water solubility
 368 of the G7–Q3G complex and the absence of the formation of a G7–QC complex (Table 2). The same
 369 phenomena were observed for the short dextrans T1 (DP = 7) and T1.5 (DP = 10) as for G7. The
 370 solubility of Q3G and QC was enhanced by 2.5- and 4.5-fold in the presence of 40 mM IMS-15/9.
 371 IMS has a chimeric structure composed of a rigid hydrophobic α -(1 \rightarrow 4)-segment and a flexible
 372 hydrophilic α -(1 \rightarrow 6)-segment. The hydrophobic segment complexes with hydrophobic compound(s),
 373 and the hydrophilic segment enhances the water solubility of the complex.



374
 375 **Fig. 4.** Phase solubility diagram for solubilization of quercetin-3-*O*- β -glucoside (Q3G) and quercetin
 376 (QC) by saccharides. **A**, Q3G; **B**, QC; \square , IMS-15/9; Δ , IMS-13/6; \blacksquare , IMS-13/3; \circ , G7; \bullet , T1; \blacktriangle , T1.5.

377 The hydrophobicity of IMS mainly originates from its α -(1 \rightarrow 4)-segment. However, both the
 378 K_c for TNS and K_s for QC flavonoids are considerably higher than those of maltodextrins (Table 2).
 379 We cannot completely explain these phenomena, but a $^1\text{H-NMR}$ analysis revealed that ethyl red
 380 interacted with the anomeric proton of the α -(1 \rightarrow 6)-segment in IMS-8/3 (Lang et al., 2014). These
 381 results suggest that the high K_c and K_s derive from the α -(1 \rightarrow 6)-segment connecting the α -(1 \rightarrow 4)- and
 382 α -(1 \rightarrow 6)-segments.

383

384 **4. Conclusions**

385 In the present study, we enzymatically synthesized IMSs containing α -(1→4)-segments of
386 various lengths from maltodextrins and investigated the IMS functions. The α -(1→6)-segment of an
387 IMS is hydrophilic and contributes to high water solubility and resistance to thermal decomposition.
388 The α -(1→4)-segment of an IMS can interact with hydrophobic ligands, and interaction depends on
389 the length of α -(1→4)-chain. The IMS chimeric nature, that is, bifunctionality of hydrophilicity and
390 hydrophobicity, enhances the aqueous solubility of compounds with low water solubility (e.g., QC
391 flavonoids), suggesting that IMSs could be extensively applied to industrial fields.

392

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402 **CRedit authorship contribution statement**

403 Weeranuch Lang: Conceptualization, Investigation, Formal analysis, Writing - original draft. Yuya
404 Kumagai: Formal analysis, Validation. Shinji Habu: Methodology, Formal analysis. Juri Sadahiro:
405 Investigation, Methodology. Takayoshi Tagami: Resources, Supervision. Masayuki Okuyama: Data
406 curation, Resources. Shinichi Kitamura: Supervision, Validation. Nobuo Sakairi: Conceptualization,
407 Supervision. Atsuo Kimura: Writing - review & editing, Resources, Supervision, Funding acquisition,
408 Project administration.

409

410 **Declaration of Competing Interest**

411 The authors have no conflicts of interest to declare.

412

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