

1 Title Gelation of cassia gum by freezing and thawing

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## Gelation of cassia gum by freezing and thawing

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### Abstract

Aqueous solution of cassia gum (CG), which is categorized as a galactomannan polysaccharide having mannose/galactose ratio=5/1, forms hydrogels by freezing and thawing. When frozen CG aqueous solution was thawed, transparent sol was separated from a turbid gel, i.e. syneresis occurred. Gel concentration ( $\{( \text{Mass of dry gel} ) / ( \text{Mass of gel} ) \} 100$ ) increased with increasing CG concentration. Viscoelastic properties of CG hydrogels formed by freezing and thawing were investigated by thermomechanical analysis (TMA) in water using an oscillation mode at 0.05 Hz. Dynamic modulus ( $E'$ ) increased from 3 kPa to ca. 5 kPa with increasing freezing rate. In contrast,  $E'$  maintained a constant value regardless of repeating number of freezing and thawing. From TMA results, it is concluded that the density of cross-linking network structure depends on the size of ice formed by freezing. At the same time, the low  $E'$  value of CG gels is ascribed to the fact that association of galactosyl side group is disturbed by the stiff chain attributed to the unsubstituted region of CG

**Keywords;** TMA, galactomannan polysaccharide, cassia gum, hydrogel, freezing and thawing

### Introduction

56

57 Cassia gum (CG), locust bean gum (LBG), tara gum (Tara-G), guar gum (GG) and  
58 fenugreek gum are categorized as galactomannan polysaccharides. Galactomannan  
59 polysaccharide is extracted from plant seeds of various plants, i.e. CG is extracted from  
60 plant seeds for *Cassia tora*, [1,2] LBG is *Ceratonia siliqua*, Tara-G is  
61 *Caesalpinia spinosa*, GG is *Cyamopsis tetragonoloba* and FG is  
62 *Trigonella foenum-graecum*. Galactomannan polysaccharides consist of a  
63 1,4- $\beta$ -D-mannose backbone and 1,6- $\alpha$ -D-galactose side chains. A structural difference  
64 among the five gums is the mannose/galactose ratio (M/G ratio), which is 5, 4, 3, 2 and  
65 1 for CG, LBG, Tara-G, GG and FG, respectively. GMs are widely used in food,  
66 pharmaceutical and cosmetic industries as stabilizers and emulsifiers since GMs hold a  
67 large amount of water [3]. Recently, the conformation of GM galactomannans with  
68 different mannose/galactose ratios has been investigated by the molecular modeling  
69 approach [4]. However, CG has received little attention since the eligibility has been  
70 recognized in recent years [5].

71 A large number of polysaccharides, such as agarose,  $\kappa$ -carrageenan and curdlan, are  
72 known to form hydrogels when aqueous solution of the above polysaccharides is cooled,  
73 by which molecular chains of polysaccharides are co-aggregated and a three  
74 dimensional network structure is established. Among various types of gelation methods,  
75 such as, cooling, heating and ionic binding, gelation of polymers by freezing and  
76 thawing is not common. A rare example is LBG [6-9]. Among synthetic polymers,  
77 poly(vinyl alcohol) is only known to form hydrogel by freezing and thawing [10-13].  
78 The above facts suggest that polymers having the capability of gel formation by  
79 freezing and thawing are rare. When the chemical structures of LBG and CG are  
80 compared, it is thought that CG may have the capability of gel formation by freezing  
81 and thawing.

82 In our previous studies, we employed thermomechanical analysis (TMA) to measure  
83 the viscoelastic properties of polysaccharide hydrogels in water [14-17]. Using the  
84 above technique, both static and dynamic mechanical properties of hydrogels at various  
85 temperatures can be measured by controlling water temperature [14]. In this study,  
86 gelation of CG by freezing and thawing is investigated by TMA in water.

87

88

89 **Experimental**

90

91 **Sample preparation**

92

93 CG in powder form was provided by Kibun Food Chemifa Co. Ltd., Japan. The  
94 commercial name is Rheo Ranger TM SR. CG was solved in deionized water at 298 K  
95 to obtain 0.1 ~ 1.5 % solutions. The solutions were annealed at 376 K for 2 h. After  
96 annealing at 378 K, 5, 10, 20, 30 or 50 ml of CG solutions were put in a 100 ml  
97 polyethylene container (49φ x 72 mm). The CG solution in PE container was directly  
98 transferred to a freezer whose temperature was 253 K and maintained for approximately  
99 18 h. Cooling rate was measured by thermometer. Obtained cooling rates were 3.0, 2.5,  
100 1.5, 1.0 and 0.7 K min<sup>-1</sup>, respectively. Frozen samples were thawed slowly at 298 K.  
101 This process took more than 6 h. The above freezing and thawing cycles are referred to  
102 as “*n*”. After freezing and thawing, the samples were stored at 298 K.

103 The gel and sol were centrifuged at 1000 rpm for 5 min. Gels were removed from the  
104 centrifugal vessel and weighed quickly. The gel ratio was calculated according to the  
105 following equation.

106

107 
$$\text{Gel ratio (g g}^{-1}\text{)} = (\text{Mass of gel}) / (\text{Mass of gel} + \text{mass of sol}) \quad (1)$$

108

109 The gel or sol was removed from the container and dried at 393 K for 10 h in an oven  
110 and dry mass of the gel was recorded. Polymer concentration was calculated according  
111 to the following equation.

112

113 
$$\text{Polymer concentration (\%)} = \{(\text{Mass of dry gel}) / (\text{Mass of gel})\} 100 \quad (2)$$

114

115 The mass of samples was measured using a Mettler Toledo microbalance (XS205).  
116 Precision was  $\pm 1.0 \times 10^{-5}$  g.

117 Concentration ratio was calculated according to the following equation.

118

119 
$$\text{Concentration ratio} = \text{Polymer concentration} / \text{Sol concentration} \quad (3)$$

120

121

122 TMA measurements in water

123

124 An SII Nano Technology Inc. thermomechanical analyser (TMA, SII Nano Technology  
125 Inc. TMA/SS 150) equipped with a newly designed sample holder was used [14]. A  
126 quartz rod with uniform cross sectional area ( $5.09 \times 10^{-5} \text{ m}^2$ ) was used as a probe. The  
127 sample holder was immersed in water whose temperature was controlled at 298 K. The  
128 gel sample was placed in a quartz sample pan with diameter 9 mm (inner diameter) and  
129 height 5 mm and immersed in 70 ml of water. The sample was compressed by quartz  
130 probe.

131 Dynamic measurements were carried out in water at 298 K using a quartz probe.  
132 Operating frequency was 0.05 Hz. Measurements were carried out for 5 min. The  
133 conditions employed were dynamic modulus ( $E'$ ) and  $\tan \delta$ . From Lissajous diagram,  $E'$   
134 and  $\tan \delta$  were obtained. 1 % CG hydrogel was used as a sample for TMA  
135 measurements.

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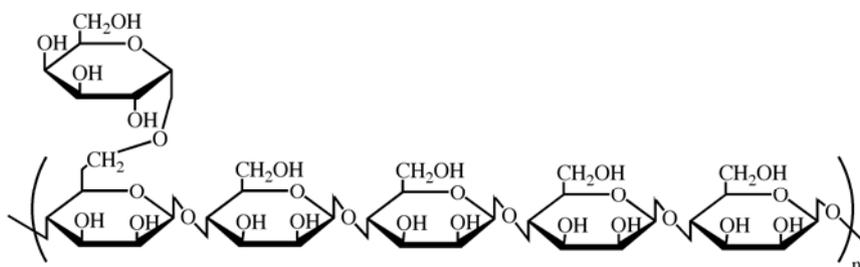
## 138 Results

139

140

141 The chemical structure of CG is shown in Fig. 1. A turbid gel was formed by  
142 freezing-thawing process of CG aqueous solution, and transparent liquid was separated  
143 from the gel at the time of thawing, i.e. syneresis occurred. When this gel is treated at a  
144 high temperature, the cross linking network decomposes.

145



146

147 **Fig. 1** Chemical structure of cassia gum

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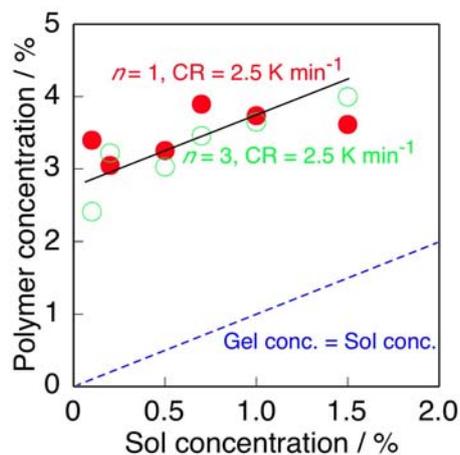
150 Effect of sol concentration on gelation

151

152 Gelation by freezing and thawing is affected by various factors such as concentration of  
153 sol, freezing rate [9], number of freezing and thawing [7] and thermal history of sol  
154 state [8]. At first, in this study, effect of sol concentration on gelation was investigated.  
155 Fig.2 shows relationships between polymer concentration in gel and sol concentration.  
156 Freezing rate was  $2.5 \text{ K min}^{-1}$ ,  $n$  was 1 or 3. The broken line shown in Fig.2 indicates  
157 that polymer concentration is assumed to be the same as sol concentration. It is clearly  
158 seen that polymer concentration is far larger than that shown in the broken line. This  
159 indicates that CG markedly condensed during freezing process. As shown in Fig.2,  
160 polymer concentration increases with increasing sol concentration. It is considered that  
161 molecular co-aggregation occurs during freezing. CG concentration in the gel state  
162 obviously increases and an excess amount of water was excluded from the gel network.

163

164



165

166 **Fig.2** Relationships between polymer concentration and sol concentration.

167 Closed circles; cooling rate (CR)=  $2.5 \text{ K min}^{-1}$ ,  $n$ (number of freezing and thawing) =1,

168 Open circles; CR= $2.5 \text{ K min}^{-1}$ .  $n=3$ . Broken line; postulated line assuming gel

169 concentration=sol concentration

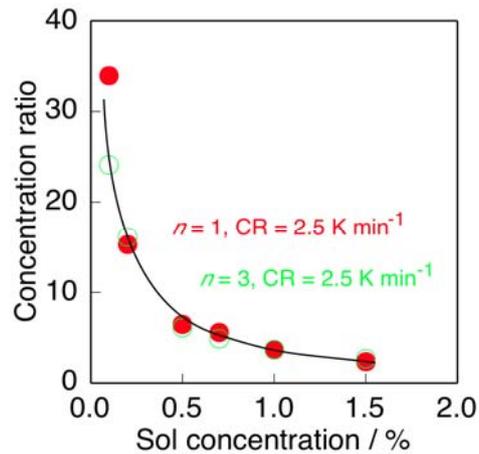
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171

172 When sol concentration is low, a large amount of water is excluded from gels in  
173 which CG mass markedly increases. Fig.3 shows the relationship between concentration  
174 ratio and sol concentration. Concentration ratio is defined in Eq. (3). As shown in Fig.3,

175 0.1 % sol is condensed ca. 35 times. Concentration ratio decreased with increasing sol  
176 concentration. This indicates that molecular co-aggregation is markedly observed when  
177 sol concentration is small.

178



179

180 **Fig.3** Relationship between concentration ratio and sol concentration.

181 Cooling rate = 2.5 K min<sup>-1</sup>, Closed circle;  $n=1$ , open circle;  $n=3$

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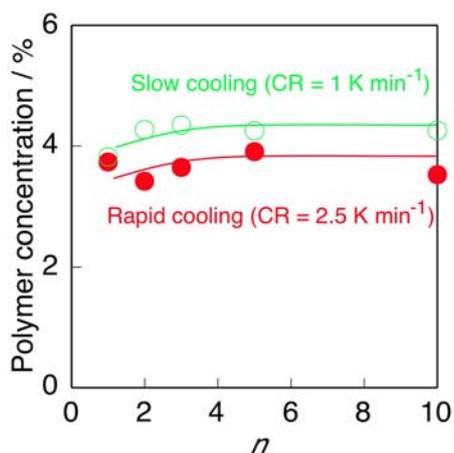
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184 Effect of number of freezing and thawing ( $n$ )

185

186 As described in the experimental section, the number of freezing thawing varied from 1  
187 to 10 times and gel concentration was measured. Fig.4 shows relationship between  
188 polymer concentration and  $n$ . The number of freezing and thawing scarcely affects the  
189 polymer concentration. This indicates that once the network structure is formed,  
190 molecular rearrangement does not occur. In contrast, it is clearly seen that cooling rate  
191 evidently affects polymer concentration, i.e. concentrations of gel formed at 1 K  
192 min<sup>-1</sup> are larger than those formed at 2.5 K min<sup>-1</sup>. Accordingly, in the latter sections of this  
193 paper, cooling rate was fixed at either  $n=1$  and  $n=3$ .

194



195

196 **Fig.4** Relationship between polymer concentration and  $n$ .

197 Concentration=1 %, Closed circle; CR=2.5 K min<sup>-1</sup>, open circle; CR=1 K min<sup>-1</sup>

198

199

200 Effect of cooling rate

201

202 Fig.5 shows polymer concentration formed at  $n=1$ (Fig.5 (a)) and formed at  $n=3$ (Fig.5  
 203 (b))as a function of sol concentration. At rapid cooling (2.5 K min<sup>-1</sup>), polymer  
 204 concentration increased with increasing sol concentration regardless of  $n$ . However, at  
 205 slow cooling, polymer concentration maintains a constant value regardless of sol  
 206 concentration. The effect of  $n$  is slightly observed when cooling is 1.0 K min<sup>-1</sup>.  
 207 Concentrations of gel formed by  $n=3$  at 1.0 K min<sup>-1</sup> are higher than those by at  $n=1$ . In  
 208 contrast, no significant difference by changing  $n$  was found for gels formed at 2.5 K  
 209 min<sup>-1</sup>. Molecular co-aggregation during freezing depends on cooling rate. This indicates  
 210 that the size of ice affects the cross-linking zone formation.

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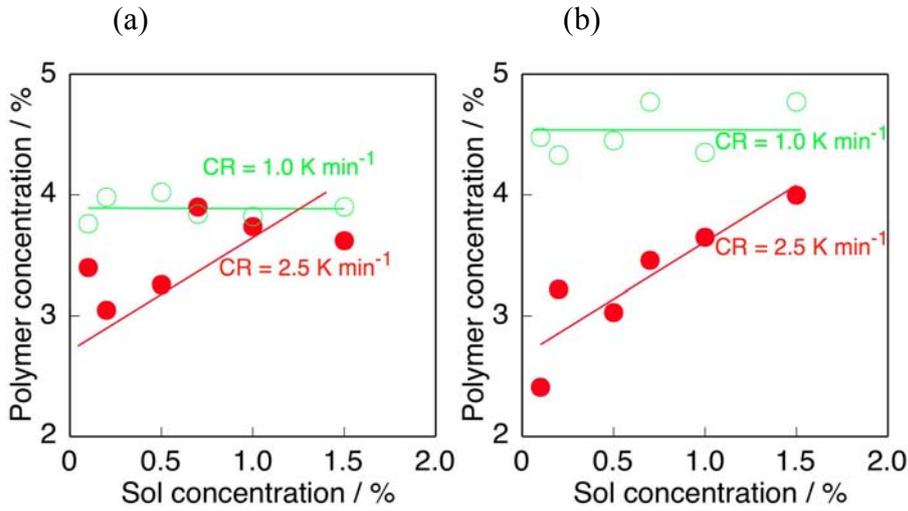
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223 **Fig.5** Relationship between polymer concentration and sol concentration.

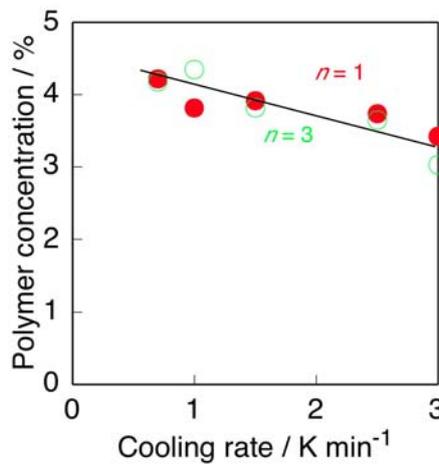
224 (a)  $n=1$ , (b)  $n=3$ . Closed circle;  $CR=2.5 \text{ K min}^{-1}$ , open circle;  $CR=1 \text{ K min}^{-1}$

225

226

227 Cooling rate varied from 0.5 to 3  $\text{K min}^{-1}$  and gel formation was examined. Fig.6  
 228 shows the relationship between polymer concentration and cooling rate.  $n$  was found to  
 229 have no effect on polymer concentration. Polymer concentration decreases with  
 230 increasing cooling rate. It is evident that cooling rate is a major factor in controlling gel  
 231 formation.

232



233

234 **Fig.6** Relationship between polymer concentration and cooling rate.

235 Sol concentration=1%, Closed circle;  $n=1$ , open circle;  $n=3$

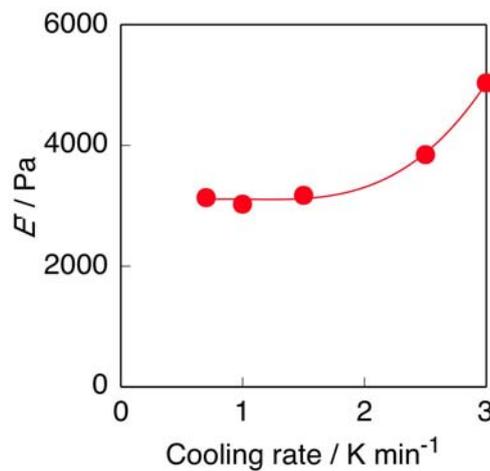
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237

238 Representative gels which formed from sol concentration = 1 %,  $n=1$ , cooling rate  
239  $0.7 \sim 3.0 \text{ K min}^{-1}$  were measured by TMA in water [9]. The sample was taken from  
240 different parts of the frozen gel and no significant difference was observed in the values  
241 of  $E'$  and  $\tan \delta$ . As reported in our previous papers, dynamic modulus  $E'$  was calculated  
242 from Lissajous diagram [14]. Fig.7 shows the relationship between  $E'$  and cooling rate.  
243  $E'$  increased from 3 kPa to ca. 5 kPa with increasing cooling rate.  $E'$  maintained a  
244 constant value regardless of  $n$ , although the figure is not shown. The cooling rate  
245 dependency of gelation is also confirmed by TMA results. A densely cross-linked  
246 network structure is formed by slow cooling.

247

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249

250 **Fig.7** Relationship between  $E'$  and cooling rate.

251 Concentration= 1 %,  $n=1$

252

253

## 254 Discussion

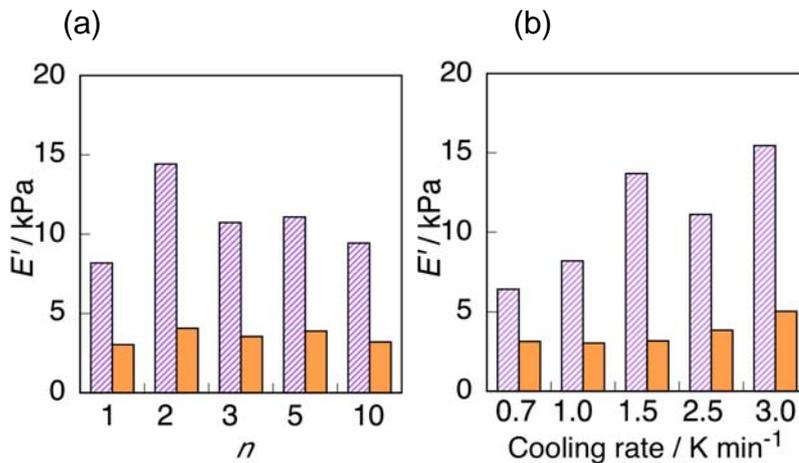
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256

257 From the above results, it was confirmed that CG forms hydrogels by freezing and  
258 thawing. At the same time, it was found that gels are formed in a limited  
259 concentration range, from 0.1 to 1.5 %. Among a series of galactomannans, LBG is  
260 known to form hydrogel by freezing and thawing [6-9]. LBG forms hydrogels in a  
261 concentration from 0.5 to 10 % under the same conditions in which CG hydrogel is

262 formed in this study. Both hydrogels are turbid, however, when cooling rate is the same,  
 263 CG excludes a large amount of water from gel as shown in Fig. 2. In contrast, LBG  
 264 showed no syneresis when concentration exceeds 3 % [9]. As shown in Fig. 4,  $n$   
 265 scarcely affects polymer concentration, although cooling rate dependency on the  
 266 gelation is noticeable. As shown in Fig. 6, polymer concentration decreases with  
 267 increasing cooling rate. Viscoelastic properties measured by TMA exhibit clearly the  
 268 characteristic features of CG gel.  $E'$  values of CG gels are compared with those of LBG  
 269 in Fig. 8.  $E'$  values of CG are far lower than those of LBG. When comparison is made  
 270 on a same scale of LBG, no marked change is observed for CG, when  $n$  and cooling rate  
 271 are varied.

272  
 273  
 274



275

276 **Fig. 8** Comparison of dynamic modulus ( $E'$ ) measured by TMA of CG and LBG.

277 Shaded bars ;LBG; solid bars ; CG.

278 (a) Concentration of CG and LBG; 1%, cooling rate; 1  $\text{K min}^{-1}$

279 (b) Concentration of CG and LBG; 1%, number of freezing and thawing ( $n$ ); 1

280

281

282 The above facts indicate that once the cross-linking zone is formed via intermolecular  
 283 association at the initial freezing process, the network structure of CG gels is  
 284 maintained and no further molecular association occurs by repeating freezing. It can be  
 285 concluded that the size of ice initially formed inside the gel is memorized and  
 286 crystallization occurs in a similar manner by successive freezing and thawing. Cooling  
 287 rate kinetically affects the aggregation of polymer chains, and therefore polymer

288 concentration decreases with increasing cooling rate. Size of ice depends on cooling rate,  
289 and quick cooling forms a small size of ice which induces a dense network structure,  
290 although this effect on CG is not marked compared with that of LBG as shown in Fig.  
291 8(b). According to computer simulations of FG, GG, Tara-G and LBG, it is thought  
292 that the galactosyl side groups have the effect of lowering the chain dimension. The  
293 intra-chain associations via H-bonding along the side groups are also ascertained [4].  
294 The galactosyl side groups associate and form a cross-linking area or junction zone.  
295 According to M/G ratio, the stiff chain attributed to the unsubstituted region of CG is  
296 longer than that of LBG. Accordingly, it is considered that once H-bonding is  
297 established, further molecular association is disturbed by stiff main chains, since the  
298 molecular chain distance between cross-linking area of CG is considerably large. The  
299 difference between LBG and CG observed in  $E'$  can be explained by mannose/galactose  
300 ratio. At the same time, it is reasonable to assume that FG, GG and Tara-G do not  
301 form hydrogels by freezing and thawing, since molecular conformation is too compact  
302 for ice crystallization which induces inter-molecular H-bonding.

303

304

## 305 **Conclusions**

306

307 CG hydrogels were prepared by freezing and thawing.  $n$  scarcely affects gelation ability.  
308 This indicates that once the network structure is formed, molecular rearrangement does  
309 not occur. In contrast, it is clearly seen that cooling rate evidently affects gelation ability.  
310  $E'$  values by measured TMA in water increased with increasing cooling rate.  $E'$  of CG  
311 are far lower than those of LBG. It is thought that the galactosyl side groups associate  
312 and form a cross-linking area or junction zone. The low  $E'$  value of CG gels is ascribed  
313 to the fact that association of galactosyl side group is disturbed by stiff chains attributed  
314 to the unsubstituted region of CG

315

316

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320

321

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324

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