

Spectroscopy Investigation on Conformational Transition of Tea Glycoconjugate from Green Tea

CHEN, Hai-Xia^{a,b}(陈海霞) ZHANG, Min^b(张民) XIE, Bi-Jun^{*a}(谢笔钧)

^a Food Science and Technology Department, Huazhong Agricultural University, Wuhan, Hubei 430070, China

^b Institute of Marine Drug and Food, Ocean University of China, Qingdao, Shandong 266003, China

The conformational transition of a new glycoconjugate, tea glycoconjugate (TGC), was investigated by spectroscopy techniques including circular dichroism (CD) and ultraviolet (UV) spectroscopy. The solution behaviors of TGC in the mediums of different temperature, pH value, and ions were compared. Results showed that the native conformation of TGC was partially ordered. The CD value and UV absorbance of TGC altered with the change of pH value, temperature, the addition of ions, and also accompanied order-disorder transition. Especially the conditions with temperature higher than the glass transition temperature ($T_g=62\text{ }^\circ\text{C}$), higher pH value or lower pH value will have the most impact on the conformation of TGC, which will destroy the hydrogen bonds between the TGC molecules. The results indicated that the outside factors play important roles on the stability of the conformation of TGC.

Keywords tea glycoconjugate, conformational transition, circular dichroism, ultraviolet spectroscopy

Introduction

The conformations of some biological macromolecules including nuclear acids, proteins, polysaccharides and glycoconjugates have been studied because the conformations are closely related to the physical and chemical properties, even the bioactivities of the compounds. Recently much attention has been paid to the secondary conformation investigation especially in aqueous solutions. The influence on conformational transition of DNA induced by cationic lipid vesicles, the effects of pH and metal ions on the conformation of bovine serum albumin in aqueous solution, the effect of temperature on the conformation of dirterbium transferring were described by previous studies.¹⁻³ The unfolding, conformational change between active sites and inactivation in SDS solutions for creatine kinase, the effects of cationic ions on the conformational properties of gellangum in aqueous solutions with and without salts, the influence of xanthan conformation on the physicochemical behavior of their mixtures, and others were observed by viscoelastic measurements, differential scanning calorimetry (DSC) and chiroptical methods, *etc.*⁴⁻⁶ Chen's group studied the conformation transition behavior of regenerated silk fibroin membranes in alcohol solution at high concentrations.⁷ The conformations of some drugs in aqueous environments were also investigated.⁸

According to the previous studies, there are several kinds of conformations of natural biological macromolecules including random coils, single helical, double

helical, three helical, *etc.* The conformational transition is dependent on the factors including the ionic strength of the solution, the nature of electrolyte, the pH value, and the temperature. For investigating the conformation, CD is a powerful tool to characterize the transitional conformations of solution samples. The time scale of CD is fast compared with molecular motion, so that conformational information needs not to be deconvoluted from dynamic contributions as is the case with NMR.⁹

Natural glycoconjugates attracts much interest because of its multiple bioactivities, pharmacological activities and safety. The conformation of glycoconjugates has stronger effects on bioactivities according to the recent studies. TGC attracted the researcher's interests because of its immunological, anti-radiation, anti-blood coagulation, anti-cancer, anti-HIV and hypoglycemic activities.^{10,11} In the field of structure studies, only the molecular weight and the monosaccharide constitutes of TGC were reported in several studies.^{12,13} But the detailed structure and conformation, especially the conformational transitions of TGC are poorly understood.

To get the optimized bioactive conformation of TGC, it is necessary to study the factors that can induce the conformational transition. In this context, our studies focused on a new TGC that was isolated and purified from low-grade green tea. The conformational transition of this polymer, under the acid, alkali and the different cationic mediums was investigated by several techniques (UV, CD). The different conformations of TGC

* E-mail: bijunxie@sina.com

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were characterized and compared. The conformational transition from the native conformation when the temperature increased was also discussed in terms of polyelectrolytic behavior.

Experimental

Materials

Lower grade green tea was purchased from Xuan'en County, Hubei Province, China. Polyamide adsorbent resin (separate components according to the polarity and group) was purchased from Nankai University Chemical Co. (Tianjin, China). Sephadex G-100 was from Pharmacia Co (Uppsala, Sweden). All other chemicals used were of analytical purity.

Preparation of TGC

Green tea powder was first extracted with ethanol three times. The extraction gave other uses and the residues were then extracted with distilled water (1 : 20 w/V, 70 °C), and crude tea polysaccharides were yielded after precipitation of ethanol and freeze-drying. Polyamide adsorption resin (3.0 cm × 60 cm i.d.) column with water as the elution was used to further remove polyphenols and polar impurities. The fraction with high polysaccharide contents was pooled and concentrated and then precipitated by ethanol. The sample was then applied to DEAE cellulose column (2.5 cm × 50 cm i.d.), giving TGC fractions with gradient NaCl solution as elution. The main fraction of TGC was further purified by Sephadex G-100 column (1.0 cm × 100 cm i.d.). The main fraction of TGC was gathered. It was homogeneous determined by the methods of column chromatography and electrophoresis. The average molecular weight of TGC was 120 kD determined by gel filtration chromatographic column of Bio-Rad G3000SW. The content of uronic acid, protein and neutral sugar was 54.4%, 3.7% and 38.1% respectively. TGC was composed of Ara, Rib, Xyl, Glc and Gal with a molar ratio of 4.9 : 2.2 : 3.1 : 1.8 : 1.0. The results of β -elimination reaction indicated that the chain of polysaccharides and protein was connected by O-linked chemical bond. According to the NMR spectroscopy analysis, the backbones of sugar residue chain in TGC were connected mainly by 1→4 and 1→6, and TGC contained α and β type glycosidic linkages. By using the technique of atomic force microscopy and laser light scattering, TGC was found to have hemi-flexible and high-branched chain. Differential scanning calorimetry measurement showed that the glass transition temperature (T_g) of TGC in water was 62 °C.

The isolation, purification and identification techniques applied to these compounds will be reported in other papers.

Preparation of the samples solutions

Sample solutions were prepared by dissolving TGC powder in distilled water. After dissolution, different solutions of salts were added to get the desired glyco-

conjugate and salt concentrations. To obtain acid and alkali mediums, TGC powder was dissolved in the acid or alkali distilled water (adjusted with 0.1 mol/L HCl and 0.1 mol/L NaOH). To detect the effects of temperature, TGC solutions were stored at the different temperature circumstances stirred for 30 min and measured by CD and UV. It was checked that the average molecular weight of TGC in different solutions was the same. So there was no thermal or acid and alkali degradation in the experimental conditions used.

Spectroscopy methods

UV-Vis spectra were measured on a scan UV-265FW spectrophotometer (Shimadzu, Japan) over a range of 190—400 nm using a 1.0 cm path length cell. CD spectra were measured using a Jasco-500 CD spectrometer (Jasco, Japan) over a range 180—350 nm with a 1.0 cm path length of rectangular quartz cell at 20 °C, controlled by a thermoelectric cell holder (Japan). Three scans were averaged per spectrum.

Results and discussion

Effect of temperature on the conformation of TGC

Because there are five neutral sugars and uronic acid existing in one repeating unit of the main chain, TGC was a polyelectrolyte. The glass transition temperature (T_g) was related to the structure properties and the state of macromolecules. The T_g of TGC was 62 °C, which indicated that the molecular chain segment motion began if the temperature was higher than 62 °C. As shown in Figure 1, there was difference in the UV-Vis spectra of TGC solution determined at 20, 60 and 100 °C respectively. The maximum peak value was increased with the raise of temperature. The conformational transition for native conformation was related to the polyelectrolytic character and molecular chiral structure of TGC. Figure 2 displayed the CD spectra when TGC was at the circumstances with the temperature of 20, 60 and 100 °C. When TGC was dissolved in distilled water, it was highly branched in a random coil state with only partial order because of the exposed hydroxyl and carboxyl groups. Its CD spectrum exhibited a strong positive Cotton effect at 258 nm corresponding to partial orderable conformation and a weak negative Cotton effect at 210 nm corresponding to the random coil and the $n \rightarrow \pi$ transition of carboxyl groups. When the temperature of solution rose to 60 °C, obvious changes of TGC conformation occurred on the spectra. After the solution temperature rose to 100 °C, the negative Cotton effect at 218 nm corresponding to the random coil and the $n \rightarrow \pi$ transition was obviously increased. The results showed that with the increase of temperature, the molecules of TGC might disaggregate each other, the electronic transition and dissymmetry increase, and also the action of hydrogen bonding decreases. Especially when the temperature was higher than T_g , the conformation changed more obviously from order to disorder with the $\Delta\epsilon$ value being increased.

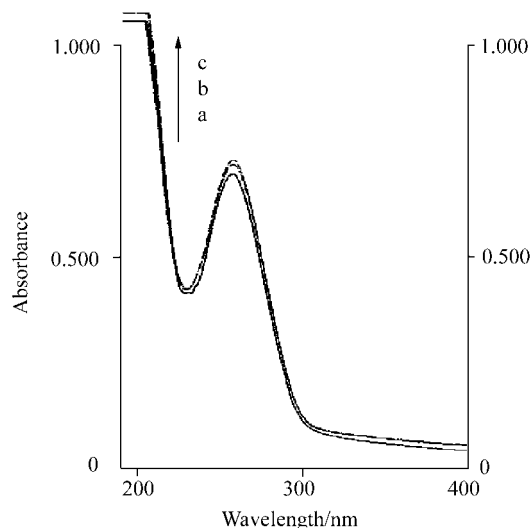


Figure 1 UV spectra of TGC at the concentration of 1.0 mg/mL at different temperature. The temperature of the solutions was (a) 20 °C, (b) 60 °C and (c) 100 °C respectively.

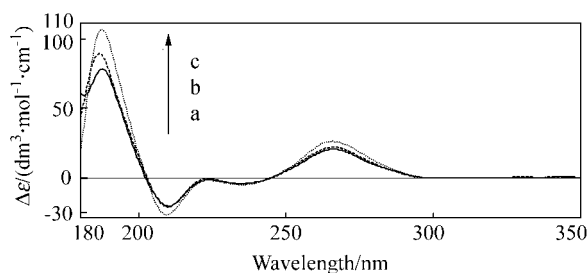


Figure 2 CD spectra of TGC at the concentration of 1.0 mg/mL at different temperature. The temperature of the solutions was (a) 20 °C, (b) 60 °C and (c) 100 °C respectively.

Effect of pH value on the conformation of TGC

The UV spectra (Figure 3) suggested that in the presence of acid or alkali the absorbance value of the

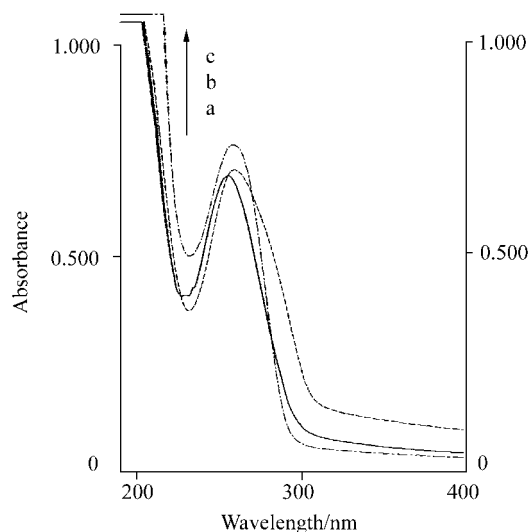


Figure 3 UV spectra of TGC at the concentration of 1.0 mg/mL at different pH value. The pH values of the solutions were (a) pH =2, (b) pH=6 and (c) pH=12 respectively.

maximum peak at 258 nm increased obviously and all the peaks shifted to long wavelength. The absorbance value of valley decreased and the absorbance value of peak increased when TGC was in an acidic environment (pH=2). While the absorbance value of peak and valley both increased in the curve of pH=12. The results indicated that with the existence of acid or alkali, the polyelectrolytic properties of TGC in solution were increased, and then the electronic transition occurred.

The CD spectra in the UV range can be used to monitor the conformational transition of biomacromolecules.¹⁴ Seen from Figure 4, the CD spectrum of TGC exhibited a positive Cotton effect at 258 nm and a negative effect at 210 nm in the solution of neutral pH. After adding acid, the solution pH value was adjusted to 2, the positive Cotton effect at 258 nm and a negative effect at 210 nm, all shifted to longer wavelength, and at the same time, the $\Delta\epsilon$ value was greatly decreased. When TGC was in an alkali environment of pH=12, the Cotton effect at 210 nm shifted to longer wavelength, a negative Cotton effect at 210 nm disappeared and a new positive Cotton effect occurred. And the $\Delta\epsilon$ value decreased greatly. The results indicated that the acid and alkali circumstance could induce the perturbations that break the symmetry of ether chromophores of TGC. There was electrostatic interaction happened in the molecule and among the molecules, which induced the conformation transition from order to disorder.

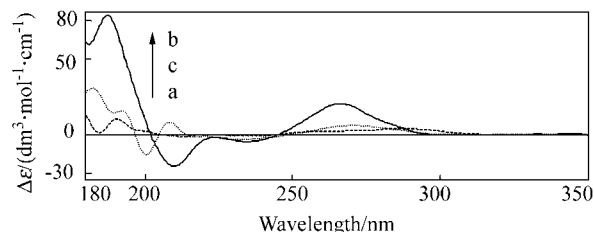


Figure 4 CD spectra of TGC at the concentration of 1.0 mg/mL at different pH value. The pH values of the solutions were (a) pH =2, (b) pH=6 and (c) pH=12 respectively.

Effect of ion addition on the conformation of TGC

The ion addition to the solution can also affect the conformation of TGC. As shown in Figure 5, the positive Cotton effect at 258 nm shifted to short wavelength weakly with the existence of calcium ion and the dissymmetry was weakened. This may be due to the electrostatic interaction between cationic calcium ion and the negatively changed uronic sites. The same phenomena were also showed after adding congo-red (Figure 6) and borate (Figure 7) to TGC solution. The decrease of dissymmetry conformation of TGC suggested that there maybe a cross-link between the ion group and TGC. This indicated that ion groups could induce the conformational transition of TGC. But the main backbone of the TGC was not changed.

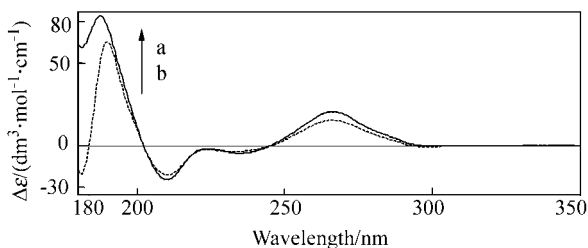


Figure 5 CD spectra of TGC at the concentration of 1.0 mg/mL in the absence (a) or presence (b) of calcium (5 mmol/L).

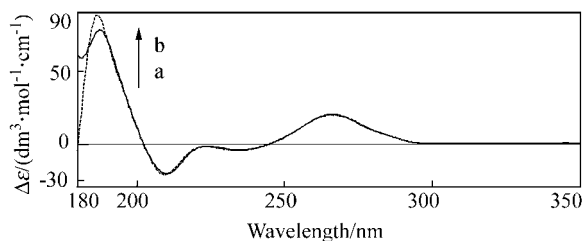


Figure 6 CD spectra of TGC at the concentration of 1.0 mg/mL in the absence (a) or presence (b) of congo-red (5 mmol/L).

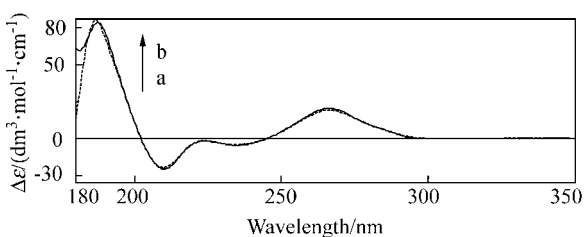


Figure 7 CD spectra of TGC at the concentration of 1.0 mg/mL in the absence (a) or presence (b) of borate (5 mmol/L).

Conclusions

CD spectra of TGC have not been reported before. The conformational transitions of TGC in solutions were detected by the technique of ultraviolet spectroscopy and circular dichroism. This work provided evidence showing that the factors including the temperature, pH and ion strength of the solution could induce the conformation alterations of TGC. When TGC was

incubated at temperatures ranging from 20 to 100 °C and solution pH value ranging from 2 to 12, conformation alterations from order to disorder were detected. The effect of solution pH value on the natural conformation of TGC was more marked in the above influence factors. The presence of ions would affect the conformation but did not change the main backbone. NMR and IR spectroscopic studies on the detailed conformational transitions of TGC are being undertaken in our laboratory. The effect of conformational transitions on the biological activity of TGC will need further investigation.

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