Effects of Tea Polysaccharide on Crystallization of Calcium Oxalate

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Abstract. The effect of tea polysaccharide (TPS) on crystal growth of calcium oxalate (CaOx) was studied. X-ray diffraction and Fourier transform infrared spectroscopy (FT-IR) results revealed that TPS inhibited crystal growth, and increased the absolute value of zeta potential on crystal surface and thus favorably inhibiting crystal aggregation. In the concentrations of 0 to 3.0 g/L, TPS only induced calcium oxalate monohydrate (COM) formation, but the crystal face strength I(011)/I(020) of COM increased. These results indicated that TPS may be a potential drug for the prevention and treatment of CaOx stones.

Introduction

Tea, originated from China, has a long history of over 4000 years, and it is also the most popular non-alcoholic beverage in Asia [1]. Tea polysaccharide (TPS) was an acid polysaccharide extracted from tea leaves [2].

Plant polysaccharides have biological activities such as anti-coagulation, anti-tumor, anti-inflammatory, antiviral and antioxidant activities [3,4]. The physicochemical properties of polysaccharides (such as molecular weight, monosaccharide composition, protein and polyphenol content), especially the content of negatively charged groups such as carboxyl group (–COOH) and sulfate group (–OSO3H), are the important factors to affect their activity. Compounds with structures containing one to two or more groups such as –OSO3H, -OH, -SH, -COOH, -PO3H2, -C=O, -NR2, and -S- will have stronger free radical scavenging ability [5].

The molecular weight and structure of tea polysaccharide were related with tea species and purification process [5, 6]. Chen et al. [7] revealed that the tea polysaccharide extracted from green tea, with the molecular weight of 120 kDa, was composed of arabinose, ribose, xylose, glucose, galactose, and uronic acid, in a molar ratio of 1.00: 0.77: 2.65: 0.88: 0.42: 2.13. Wang et al. [8] extracted a water-soluble polysaccharide (7WA), with an average molecular weight of 7.1×10^4 Da from green tea. 7WA mainly contained arabinose and galactose in the molar ratio of 1.0:0.96, and it possessed a backbone consisting of a 1,3- and 1, 6-linked galactopyranosyl residues, with branches attached to O-3 of 1, 6-linked galactose residues, and O-4 and O-6 of 1, 3-linked galactose residues.

Wang et al. [9] gained a polysaccharide component (ZTPs) from green tea with a molecular weight of 8000 Da by hot water extraction and ethanol precipitation. ZTPs was composed of mannose, ribose, rhamose, glucuronic acid, galacturonic acid, glucose, xylose, galactose, arabinose and fucose, with molar percentages of 4.3%, 1.4%, 4.1%, 2.6%, 3.0%, 31.4%, 4.6%, 21.8%, 23.5% and 3.3%, respectively. Zhou et al. [10] extracted a tea polysaccharide from green tea, which was mainly consisted of rhamose, glucose and galactose by β-(1, 3)-glycosidic
bond. All above three monosaccharide residues is connected to arabinose via β-(1, 2), β-(1, 3), β-(2, 3) glycosidic bond. However, the xylose exists at the terminal end of either the main chain or the branch chain with β1→ linkage. Tea polysaccharide aqueous solution was existed with ordered spiral structure.

Oxidative injury is one of the main factors that cause many diseases. The formation of kidney stone is related to the oxidative damage of kidney epithelial cells [11]. On account of that tea polysaccharide has good antioxidant activity, which can reduce the oxidative damage of the cells [12]. Therefore, tea polysaccharide may be used to reduce the incidence of kidney stone and prevent the formation of kidney stone.

**Experiments in Details**

**Reagents and Apparatus**

Sodium oxalate (Na$_2$Ox) and calcium chloride (CaCl$_2$) was analytically pure. Other chemical reagents were analytical grade and purchased from Guangzhou chemical reagent company (Guangzhou, China). Tea polysaccharide was provided by Shanxi Ciyuan Biology Co., Ltd.

The apparatus included D/max 2400-X ray powder diffractometer (Rigaku, Japan), Fourier transform infrared spectrometer (Nicolet, American), and Zetasizer 300HS nanoparticle size-Zeta potential analyzer (Malvern, England).

**Crystallization of CaOx**

CaCl$_2$ solution and TPS solution were mixed and magnetically stirred for 10 min. Sodium oxalate was then added to the mixture. The final concentrations of Ca$^{2+}$ and Ox$^{2-}$ were both 10 mM, and the polysaccharide concentrations varied from 0 g/L to 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g/L. After 10 min, the mixture was maintained at 37 °C for 2 h, and the suspension was centrifuged. The bottom deposits of CaOx were dried for component analysis through X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR).

**Zeta Potential Measurement of CaOx Crystals**

A certain amount of CaOx crystals (10.0 mg) was dispersed in 30 mL double distilled water. After 10 min of ultrasonication, the zeta potential was detected with a Zetasizer Nano ZS90 apparatus at 25 ºC.

**Results and Discussion**

**XRD Spectra of Calcium Oxalate Crystals in the Presence of Tea Polysaccharide (TPS)**

Fig. 1 showed the XRD patterns of CaOx crystals formed in presence of different concentrations of TPS. Only calcium oxalate monohydrate (COM) crystal formed. The main diffraction peaks of COM appeared at d=0.591, 0.364, 0.296 and 0.235 nm, which attributed to the (001), (020), (202) and (213) planes of COM crystal, respectively. After addition of TPS of concentration of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g/L, respectively, only COM were induced (Fig. 1b-1g), and no calcium oxalate dihydrate (COD) was formed.

TPS easily absorbed on positively-charged (001) plane of COM. When TPS was added, the diffraction peak intensity of (001) plane was gradually strengthened but that of (020) plane was weakened with increasing TPS concentration (Fig. 2). It was attributed to higher released
adsorption energy when negatively-charged -COOH was absorbed on positively-charged (01 1) plane [13]. In addition, growth rate of (01 1) face will be slower after TPS was absorbed on it. Since crystal plane with slow growth rate disappeared slowly, diffraction peak intensity of (01 1) plane of COM became gradually stronger, but diffraction peak intensity of the other crystal planes such as (020) plane became gradually weaker. This effect was more obvious when the TPS concentration increased.

Figure 1. XRD patterns of CaOx crystals formed in presence of different concentrations of TPS respectively. (a) 0; (b) 0.5; (c) 1.0; (d) 1.5; (e) 2.0; (f) 2.5; (g) 3.0 g/L.

Figure 2. (01 1)/(020) crystal face ratio of COM induced by different concentrations of TPS.

**FT-IR Spectra of Calcium Oxalate Crystals in the Presence of TPS**

The FT-IR spectra of CaOx crystals formed in the presence of different concentrations of TPS were determined and shown in Fig. 3. The asymmetrical stretching vibration \( \nu_{as} (\text{COO}^-) \) of carbonyl appeared at about 1616 cm\(^{-1}\), whereas the \( \nu_{s} (\text{COO}^-) \) appeared at about 1322 cm\(^{-1}\), both indicating the presence of COM crystals [14].
Effect of Different TPS on Zeta Potential of Crystal

The Zeta potential can be used as a measurement of repulsive force between particles. The higher absolute value of Zeta potential on crystal surface suggested higher charge density of crystal surface, larger repulsive force among crystals, greater dispersion of crystal in solution, and worse crystal aggregation, which were more conducive to inhibit aggregation of crystals.

The Zeta potentials of formed CaOx crystal induced by TPS were shown in Fig. 4. It can be seen that the Zeta potential was gradually more negative as TPS concentration increased, suggesting that more negative charged ions adsorbed on crystal surface.

![Figure 3. FT-IR spectra of CaOx crystals in presence of different concentrations of TPS respectively. (a) 0; (b) 0.5; (c) 1.0; (d) 1.5; (e) 2.0; (f) 2.5; (g) 3.0 g/L.](image)

![Figure 4. Zeta potential of CaOx crystals induced by different concentrations of TPS.](image)
Effects of TPS on CaOx Precipitates

The amount of CaOx precipitation induced by different concentrations of TPS was listed in Table 1. As TPS concentration increased, the mass of induced CaOx crystals precipitate reduced. The above results revealed that the Ca$^{2+}$ chelating ability strengthened as TPS concentration increased. The growth of CaOx crystal was inhibited, which was conducive to inhibit oxalate stone formation.

Table 1. The amount of CaOx precipitation induced by different concentrations of TPS.

<table>
<thead>
<tr>
<th>Concentrations of TPS / g/L</th>
<th>0</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
<th>7.0</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitation amount / g</td>
<td>0.201</td>
<td>0.152</td>
<td>0.142</td>
<td>0.136</td>
<td>0.134</td>
<td>0.125</td>
<td>0.121</td>
<td>0.118</td>
<td>0.106</td>
<td>0.097</td>
</tr>
</tbody>
</table>

Conclusions

TPS has inhibition effect on CaOx crystal generation. Moreover, as concentrations of TPS increased, quantity of formed CaOx crystal decreased, and Zeta potential of crystal surface was more negative, aggregation degree of crystal was reduced, all of which are helpful to inhibit the formation of CaOx stone. Therefore, TPS may be a potential anti-stone drug.

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References


