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Outer-surface adduct formation and selective separation of *cis*-type gallated and *cis*-type non-gallated catechins using cucurbit[7]uril†

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We found that *cis*-type gallated and non-gallated catechins form low water solubility outer-surface adducts with cucurbit[7]uril in aqueous solution, with *cis*-type gallated catechins showing a higher tendency to precipitate as outer-surface adducts. By exploiting this phenomenon, we achieved the selective separation of *cis*-type gallated and non-gallated catechins in solution.

Cucurbit[*n*]urils (CB[*n*]s) are macrocyclic compounds in which *n* glycoluril units are polymerized through methylene bridges, forming different ring sizes such as CB[5], CB[6], CB[7], CB[8], and CB[10], depending on the degree of polymerization (Fig. 1).[1] CB[*n*]s are known to encapsulate neutral or cationic compounds within their cavities through hydrophobic effects, van der Waals forces, and electrostatic interactions, forming inclusion complexes. This property has been the focus of extensive research worldwide.[2] Among the CB[*n*] family, CB[7] is particularly notable for its ability to encapsulate molecules of a similar size to β-cyclodextrin (βCD), making it promising for pharmaceutical and food applications owing to its high water solubility and low toxicity.[3] In contrast, it is known that certain organic compounds with electron-rich aromatic rings and some anions can interact with the outer surface of CB[*n*], forming outer-surface adducts through π–π interactions with the carbonyl groups, or CH–π and electrostatic interactions with the methine hydrogens.[4] This outer-surface adduct formation is driven by the electrostatic potential of CB[*n*], which is negative around the carbonyl groups and positive around the five-membered rings.[4,5] The ability to form outer-surface adducts without encapsulating molecules in the CB[*n*] cavity broadens the range of potential applications, as it removes size constraints on the target organic compounds. Despite this, CB[*n*] outer-surface adducts have been far less studied than their

inclusion counterparts. Existing studies have largely focused on structural analyses or applications of outer-surface adducts involving interactions with ions,[4,6] with only a few examples of outer-surface adducts formed solely from organic compounds.[7,8] To expand the application potential of CB[*n*]s, we explored and developed new outer-surface adducts composed exclusively of CB[*n*] and organic compounds.

First, we focused on catechins, which are polyphenols found in tea leaves, as potential organic compounds that can form outer-surface adducts with CB[*n*]. Catechins contain multiple electron-rich aromatic rings and have been reported to form complexes with compounds like caffeine and nicotinamide through π–π and CH–π interactions.[9] Moreover, catechins occur naturally and have a wide range of synthesized derivatives, making them ideal candidates for investigating their tendency and mechanisms for forming outer-surface adducts with CB[*n*].[10] In this study, we used four major catechins found in tea leaves: the *cis*-type gallated catechins with a galloyl group ((−)-epigallocatechin-3-*O*-gallate (EGCg) and (−)-epicatechin-3-*O*-gallate (ECg)) and the *cis*-type non-gallated catechins without a galloyl group ((−)-epigallocatechin (EGC) and (−)-epicatechin (EC)) (Fig. 1).[11] Because outer-surface adduct formation between *cis*-type catechins and CB[*n*] is expected to occur in
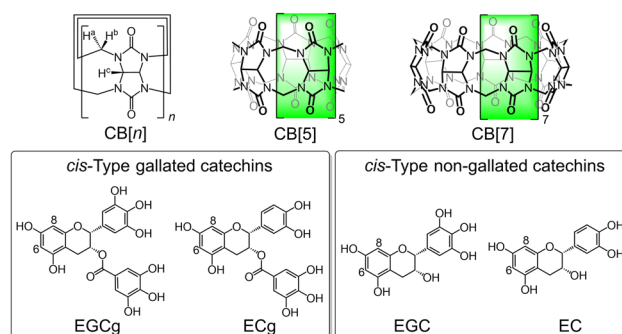
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Fig. 1 Chemical structures of CB[*n*], CB[5], CB[7], and *cis*-type catechins.

aqueous solution, we selected CB[7], the most water-soluble CB[n], and used CB[5], another water-soluble CB[n], for comparison (Fig. 1).

Fig. 2 shows the solution appearance as the equivalent amount of CB[7] was increased from 0 to 6 while maintaining a constant concentration of cis-type catechins (EGCg, EGC, ECg, and EC: 0.5 mM) at 293 K. For cis-type gallated catechins (EGCg or ECg), the solutions became turbid starting at 0.5 equivalents of CB[7] and remained turbid up to 6 equivalents. In contrast, solutions of cis-type non-gallated catechins (EGC or EC) remained clear up to 2 equivalents of CB[7], becoming slightly turbid at 4 equivalents and showing noticeable turbidity at 6 equivalents. The mass of the precipitate collected by filtering these solutions increased with the number of CB[7] equivalents for all cis-type catechins (Fig. S1, ESI†). However, no precipitate was observed when 0.5–2 equivalents of CB[7] were added to the cis-type non-gallated catechins. Since CB[7] remains acidic even after purification, this precipitation phenomenon was considered as possibly due to the aggregation of cis-type catechins caused by a decrease in pH with increasing CB[7] concentration. However, even when cis-type catechins were present under acidic conditions without CB[7], the turbidities were not observed (Fig. S2, ESI†). Typically, CB[7] is known to encapsulate compounds in its cavity, thereby enhancing their water solubility. However, the unexpected turbidity observed upon the addition of CB[7] was an interesting finding.

It has been reported that the Congo red dye can be removed from aqueous solution as a solid by forming outer surface interactions with CB[6] or CB[7], resulting in a supramolecular aggregate when these CB[n] are added.[8] If the turbidity observed from mixing cis-type catechins with CB[7] is attributed to a decrease in water solubility caused by interactions outside CB[7] and the formation of outer-surface adducts, then two conditions are expected: (1) cis-type catechins and CB[7] should be present in the precipitate, and (2) cis-type catechins and CB[7] in the solution should not form inclusion complexes. To verify these points, precipitate compositions were investigated.

Precipitates obtained after filtering turbid mixed solutions containing EGCg and CB[7] or ECg and CB[7] (at CB[7] equivalents of 0.5, 1, 2, 4, and 6), as well as precipitates obtained after filtering turbid mixed solutions containing EGC and CB[7] or EC and CB[7] (at 6 equivalents of CB[7]) were analyzed using ^{1}H NMR. It was found that these precipitates contained both cis-type catechins and CB[7] (Fig. 3 and Fig. S3, S4, ESI†). Additionally, ^{1}H NMR spectra containing EGCg or EGC clearly showed splitting of the proton signals for H[b] and H[c], which are directed outward from CB[7] (Fig. 3 and Fig. S3, S4, ESI†). This

suggests that EGCg or EGC forms CH–π interactions or hydrogen bonds on the outer side of CB[7]. For EGCg and ECg, the molar ratio of CB[7] to cis-type gallated catechins in the precipitate increased with increasing CB[7] (Table S1, ESI†). In contrast, for EGC and EC, the molar ratios of CB[7] to cis-type non-gallated catechins in the precipitate were 1.33 and 1.36, respectively (Table S1, ESI†).

The formation of inclusion complexes by CB[7] is assessed by observing shifts in proton signals using ^{1}H NMR. To assess this, heavy aqueous solutions were prepared by adding 1 equivalent of CB[7] to a constant concentration of cis-type catechins (EGCg, ECg, EGC, and EC: 0.5 mM). For turbid solutions, saturated heavy aqueous solutions were prepared after centrifugation to remove precipitates. The chemical shift values of the proton signals were compared between the solutions containing only cis-type catechins and those containing both cis-type catechins and CB[7]. The results showed no changes in the proton signals of the cis-type catechins (Fig. S5, ESI†).

To further confirm that the observed phenomenon was due to the formation of outer-surface adducts between cis-type catechins and CB[7], a similar experiment was conducted using CB[5], which cannot encapsulate cis-type catechins owing to its smaller ring diameter compared to CB[7]. As expected, adding CB[5] to cis-type gallated catechins produced turbidity, similar to the effect with CB[7]. However, for cis-type non-gallated catechins, turbidity was only observed when more than 4 equivalents of CB[5] were added (Fig. S6, ESI†). The mass of the precipitate obtained by filtering these solutions increased with the number of CB[5] equivalents for all cis-type catechins (Fig. S7, ESI†). Further ^{1}H NMR analysis of the precipitates revealed that they contained both cis-type catechins and CB[5] (Fig. S8 and S9, ESI†). The results from experiments with CB[5] were highly similar to those obtained with CB[7]. While βCD is known to encapsulate cis-type catechins, and CB[7] has a cavity similar to that of βCD, the results with CB[5] indicate that cis-type gallated and cis-type non-gallated catechins form outer-surface adducts with CB[7], resulting in a less water-soluble complex.

We observed that the appearance of the solution and the mass of the precipitate varied with increasing equivalents of CB[7] for cis-type gallated versus cis-type non-gallated catechins (Fig. 2 and Fig. S1, ESI†). This variation may be attributed to cis-type gallated catechins, with their galloyl groups, being more likely to form outer-surface adducts with CB[7], or it could indicate that the outer-surface adducts formed by cis-type gallated catechins and CB[7] are less water-soluble than those
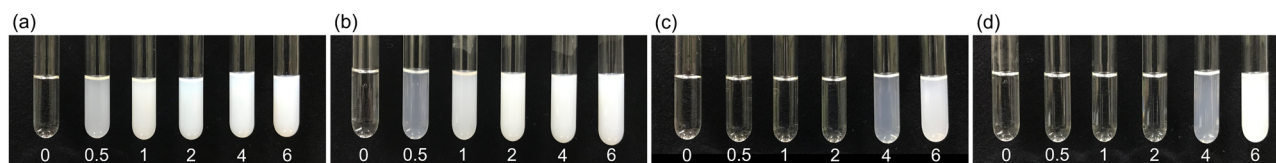

Fig. 2 Appearance of the aqueous solutions of cis-type catechins mixed with CB[7] at 293 K. (a) EGCg and CB[7], (b) ECg and CB[7], (c) EGC and CB[7], and (d) EC and CB[7]. The numbers in the image indicate the equivalents of CB[7] relative to cis-type catechins.
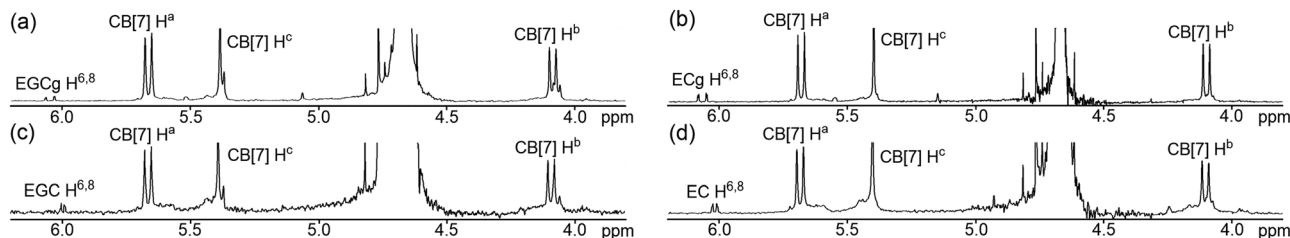
Fig. 3 ^{1}H NMR spectra of the precipitates formed by mixing *cis*-type catechins with CB[7] (6 equivalents of CB[7]): (a) EGCg and CB[7], (b) ECg and CB[7], (c) EGC and CB[7], and (d) EC and CB[7].

formed by *cis*-type non-gallated catechins. Noting this difference, we explored whether CB[7] outer-surface adduct formation could be applied to selectively separate *cis*-type gallated catechins from *cis*-type non-gallated catechins. To determine the conditions for selective separation, we investigated the residue rate of *cis*-type catechins in the filtrate of turbid solutions with varying equivalents of CB[7] (0–6 equivalents) at temperatures ranging from 278 to 298 K. The residue rate of *cis*-type gallated catechins (EGCg and ECg) decreased as the number of CB[7] equivalents increased and the temperature decreased (Fig. 4 and Tables S2, S3, ESI†). Notably, when the CB[7] equivalent exceeded 4 and the temperature was below 283 K, the residue rates of EGCg and ECg fell below 5.0%, indicating that these catechins were nearly completely removed from the solution. In contrast, the residue rates of *cis*-type non-gallated catechins (EGC and EC) did not decrease as considerably when CB[7] was added to solutions containing EGC or EC. Specifically, their residue rates remained above 95% when the CB[7] equivalent was below 4 and the temperature was above 293 K, indicating that EGC and EC remained largely in the solution (Fig. 4 and Tables S4, S5, ESI†). As a supplementary analysis, CB[5] was also examined. The residue rate of *cis*-type gallated catechins was lower with CB[7] than with CB[5], while for *cis*-type non-gallated catechins, CB[7] showed a higher residue rate than CB[5] (Fig. S10, ESI†). CB[7] was more effective than CB[5] for the selective separation of *cis*-type gallated and non-gallated catechins, and it is thought that the ring size of CB[n] is strongly related to the complex formation with *cis*-type catechins.

Based on these results, achieving selective separation of *cis*-type gallated and *cis*-type non-gallated catechins with CB[7] requires conditions that maximize the differences in their residue rates. Therefore, the selective separation of these catechins using CB[7] was investigated under the top three conditions—(i) 293 K with 4 equivalents of CB[7], (ii) 298 K with 4 equivalents of CB[7], and (iii) 298 K with 6 equivalents of CB[7]—which showed notable differences in the results shown in Fig. 4 (Table S6, ESI†). The experiments were performed in a solution containing 0.25 mM each of EGCg, ECg, EGC, and EC. Residue rates of the *cis*-type catechins were determined from the HPLC peak area values of the catechins in the solution before and after adding CB[7] (Fig. S11 and Table S7, ESI†). Under condition (i), the residue rates of EGCg and ECg were 2.9% ± 0.4% and 6.3% ± 1.1%, respectively; under condition (ii), they were 7.3% ± 0.3% and 9.8% ± 0.5%; and under condition (iii), they were 4.0% ± 0.1% and 6.1% ± 0.5%, respectively (Fig. 5, and Table S8, ESI†). In contrast, the residue rates of EGC and EC were 77.2% ± 1.7% and 78.8% ± 4.4% under condition (i), 71.7% ± 2.5% and 77.1% ± 1.6% under condition (ii), and 61.5% ± 1.9% and 70.0% ± 1.5% under condition (iii) (Fig. 5 and Table S8, ESI†). Condition (i) effectively allows for the selective removal of EGCg and ECg from the solution, although some EGC and EC are also removed (Fig. 6a). Additionally, after centrifuging the turbid solution obtained under condition (i), HPLC analysis of the resulting precipitate confirmed that it mainly contained EGCg and ECg (Fig. 6b). This demonstrates that even when *cis*-type gallated and non-gallated catechins are present together, they can be selectively separated using condition (i) (293 K and 4 equivalents of CB[7] relative to *cis*-type gallated catechins), with the *cis*-type gallated catechins forming a precipitate as a CB[7] outer-surface adduct while the *cis*-type non-gallated catechins remain dissolved in the solution.

Most research on CB[n] has focused on inclusion complexes involving their cavities, with relatively few studies addressing outer-surface adducts. In this study, we discovered that *cis*-type
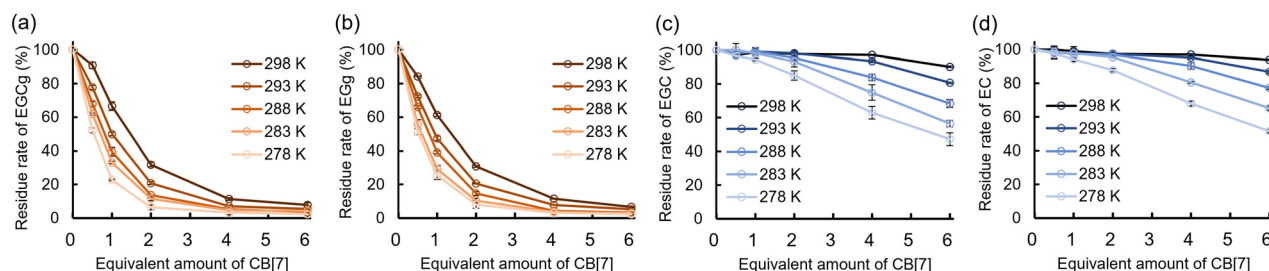

Fig. 4 Changes in residue rates of *cis*-type catechins with increasing equivalents of CB[7] at a constant concentration of *cis*-type catechins (0.5 mM) at temperatures of 278, 283, 288, 293, and 298 K (n = 3): (a) EGCg and CB[7], (b) ECg and CB[7], (c) EGC and CB[7], and (d) EC and CB[7].
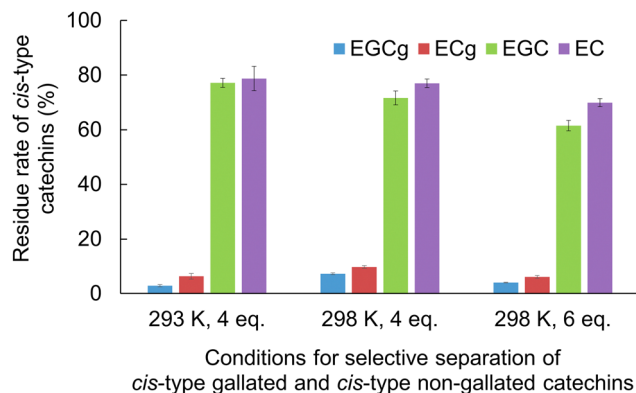
Fig. 5 Residue rates of *cis*-type catechins under different conditions of temperature and CB[7] equivalents.
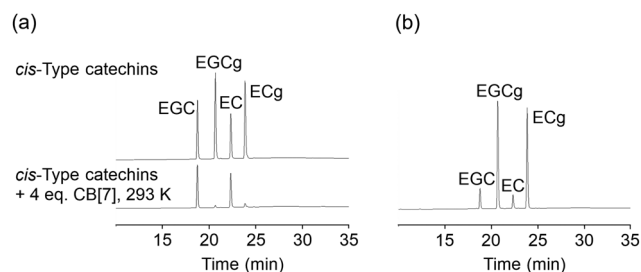

Fig. 6 HPLC chromatograms of *cis*-type catechins in (a) solution after precipitate removal and (b) solution with the dissolved precipitate.

catechins from tea leaves formed outer-surface adducts with CB[7], and this interaction could be used for the selective separation of *cis*-type gallated and non-gallated catechins. Although further investigation using computational chemistry is needed, based on the results of previous studies by Ishizu *et al.* and Charlton *et al.* and Table S1 (ESI†), it is thought that the precipitation that occurs from CB[7] and *cis*-type catechins is due to the outer-surface adduct formations through CH–π interactions and hydrogen bonds, and further aggregation.[12] Although our study focused on *cis*-type catechins, CB[7] is believed to preferentially form water-insoluble complexes with compounds containing galloyl groups. Thus, CB[7] is promising for the isolation and purification of polyphenols with galloyl groups from plants and foods, a task that has proven challenging with traditional methods. It can be inferred that similar to *cis*-type catechins, any compound with electron-rich aromatic rings may also form an outer-surface adduct with CB[7]. Our findings provide a novel example and methodology for the outer-surface adduct formation involving CB[7], representing a considerable advancement that will broaden the potential applications of CB[7] beyond its traditional use in inclusion complexes.

Conflicts of interest

There are no conflicts to declare.

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Data availability

The data supporting this article have been included as part of the ESI.†