Research Article

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Study on the interaction between catechin and cholesterol by the density functional theory

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Abstract: Catechin – a natural polyphenol substance – has excellent antioxidant properties for the treatment of diseases, especially for cholesterol lowering. Catechin can reduce cholesterol content in micelles by forming insoluble precipitation with cholesterol, thereby reducing the absorption of cholesterol in the intestine. In this study, to better understand the molecular mechanism of catechin and cholesterol, we studied the interaction between typical catechins and cholesterol by the density functional theory. Results show that the adsorption energies between the four catechins and cholesterol are obviously stronger than that of cholesterol themselves, indicating that catechin has an advantage in reducing cholesterol micelle formation. Moreover, it is found that the molecular interactions of the complexes are mainly due to charge transfer of the aromatic rings of the catechins as well as the hydrogen bond interactions. Unlike the intuitive understanding of a complex formed by hydrogen bond interaction, which is positively correlated with the number of hydrogen bonds, the most stable complexes (epicatechin-cholesterol or epigallocatechin-cholesterol) have only one but stronger hydrogen bond, due to charge transfer of the aromatic rings of catechins.

Keywords: catechin, cholesterol-lowering, density functional theory, micelle

1 Introduction

Mammals, including humans, require cholesterol for normal metabolism. Cholesterol is an indispensable and

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important substance in animal tissue cells, which not only participates in the formation of cell membranes but is also a raw material for the synthesis of bile acids, vitamin D and steroid hormones. Nevertheless, because of the strong correlation between the level of total cholesterol in the blood and the incidence of coronary heart disease, cholesterol has become notorious due to unhealthy diet, for many years [1].

At present, many drugs are available to reduce cholesterol. Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase, also commonly known as statins, are the most effective and the most widely used cholesterollowering drugs [2]. However, current medicines including statins and cholestyramine in the treatment of cholesterollowering suffer from some side effects [3]. Therefore, there is a growing interest in using functional foods and nutraceuticals to manage the cholesterol lowering, such as tartary buckwheat flour [4], lignans [5] and catechins [6].

Since ancient times tea has been used as a cure for many ailments and features prominently in traditional Chinese medicine. In fact, the alleged health benefits of tea are usually believed to be derived from the high concentration of polyphenol flavonoids, including the group known as catechins [7]. Catechins mainly include four monomers: epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG) and epicatechin (EC) [8,9], as shown in Figure 1. A number of studies have suggested that catechins are beneficial to combat depression [10,11], influenza [12], HIV [13,14] and obesity [15]. Catechins have been shown to inhibit macrophage nitric oxide generation and autophagy [16], to improve learning and memory deficits [17], to alleviate ischemia/reperfusioninduced cerebral injury [18], to enhance the antioxidant capacity of plasma [19] and especially to reduce cholesterol [20-22]. The study found that a diet supplemented with EGCG increased cholesterol levels in rats' feces [23,24]. Based on this, some researchers have found that EGCG and cholesterol can form insoluble coprecipitations through in vitro experiments [25]. In addition, in vivo experiments have shown that catechins can reduce intestinal absorption by reducing the solubility of cholesterol in mixed micelles [26]. It indicates that consumption of catechin can weaken

358 — Kaiwen Zheng et al. DE GRUYTER

Figure 1: Chemical structures of four catechins (A represents A-ring, B represents B-ring, C represents C-ring and D represents D-ring).

intestinal absorption through interacting with cholesterol intake, thereby achieving cholesterol lowering to some extent [24–27]. However, compared to a large number of applied studies, few theoretical research studies have explored the interaction patterns between cholesterol and catechins, such as the analysis of the pharmacological activity sites of catechins.

In this study, we performed density functional theory (DFT) simulations to study the interaction of typical catechins and cholesterol and analyze the adsorption sites between them. Our results demonstrated that the adsorption sites of the most stable complexes of EC-Chol and EGC-Chol are all on the A-ring, while the adsorption sites of ECG-Chol and EGCG-Chol are on the B-ring and gallic acid ester. All the adsorption energies of the complexes are obviously stronger than that of Chol-Chol themselves, indicating that catechins have an advantage in reducing cholesterol micelle formation. The analysis of molecular orbitals, natural bond orbital (NBO) and partial density of states (PDOS) of the complexes reveals that the molecular interactions of the complexes are mainly due to charge transfer of the aromatic ring of the catechins as well as the hydrogen bond interactions between catechins and cholesterol. In addition, the adsorption energy of EC-Chol or EGC-Chol is not positively correlated with the number of hydrogen bonds, which is unlike the intuitive understanding of a complex formed by the interaction of hydrogen bonds. These results suggest that the aromatic rings of the catechins enhance the adjacent hydrogen bonding through charge transfer, illustrating the interaction between catechins and cholesterol.

2 Computational methodology

After considering the advantages, disadvantages and practicability of many calculation methods, we find that B3LYP series methods are still used by many researchers [28-31]. Therefore, we performed DFT calculations using the B3LYP as an exchange correlation functional to study the intermolecular interactions. The electron wave functions in the Gaussian function basis were expanded. For geometry optimizations of cholesterol, catechins and their corresponding catechin-cholesterol complexes, double-C basis was employed and a p-polarization function was added for every nonhydrogen atom (marked with 6-31G(d)) [32]. The optimized stationary points were identified as minima or first-order saddle points via the Berny algorithm. Vibrational frequencies were computed at the same level of theory, with the aim to characterize all structures as minima and saddle points. Furthermore, the NBO, IR and PDOS of the complexes were analyzed [33] to obtain an insight into the bonding features and the nature of the intermolecular interactions. The energies of the catechin-cholesterol complexes were calculated with the zero-point energy (ZPE) correction [34]. All calculations were performed by using Gaussian 09 software package (Gaussian, Inc., Wallingford, CT, USA) [35].

The most stable catechin–cholesterol complexes were obtained as follows: four typical catechins (EC, EGC, ECG and EGCG) were chosen to analyze the interaction with cholesterol. The optimized molecular structures of catechins are shown in Figure S1. As an example, the optimized EC was compared with the experimental X-ray data [36] from the three aspects of bond distances, bond angles and torsion angles. As shown in Table S1, our calculations are consistent with the experimental data, indicating that the catechin structures optimized by the B3LYP/6-31G(d) method are reliable and accurate.

To test the most stable complexes of catechin-cholesterol, we first designed 10 EC-Chol complexes with different adsorption sites, then optimized them at B3LYP/3-21G and obtained the energies of these complexes. Compared with the lowest energy complex, the complexes within an energy difference of 10 kcal/mol were relatively stable and considered. These complexes were optimized further at B3LYP/6-31G(d) to increase the accuracy of the calculation. Vibrational frequencies were also calculated, which showed no imaginary frequency, indicating that the optimized structures are stable. To obtain ZPE, the single-point energies of the final complexes were calculated at the level of B3LYP/6-311+G(d,p)//B3LYP/6-31G(d). Thus, the most stable complex of EC-Chol can be predicted by comparing the zero correction energy. Similarly, we have obtained the complexes

of EGC-Chol, ECG-Chol, EGCG-Chol and Chol-Chol with the lowest energy. In addition, the effect of dispersion on the complexes was discussed by using the B3LYP-D3 method.

Ethical approval: The conducted research is not related to either human or animal use.

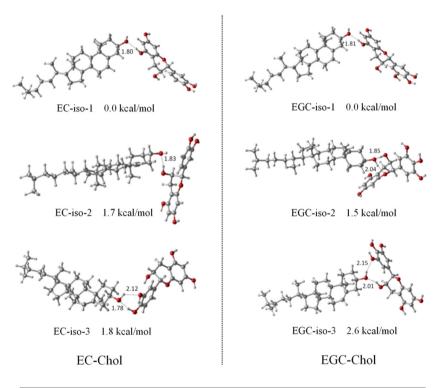
3 Results and discussion

3.1 Structures and energy of catechin-cholesterol complexes

The structures of EC-Chol complexes with energy difference within 5 kcal/mol are shown in Figure 2. In the most

stable structure (EC-iso-1), a hydrogen bond with a bond length of 1.80 Å is formed between hydroxyl hydrogen on the A-ring of EC and hydroxyl oxygen of cholesterol. The energy difference of the EC-iso-2 relative to the EC-iso-1 is 1.7 kcal/mol, in which a hydrogen bond with a bond length of 1.83 Å is formed between the hydroxyl oxygen of cholesterol and hydroxyl hydrogen on the C-ring of EC. The energy difference of the EC-iso-3 is 1.8 kcal/mol, in which a hydrogen bond with a bond length of 1.78 Å is formed between cholesterol and hydroxyl hydrogen on the B-ring of EC and another bond with a bond length of 2.12 Å is formed between hydroxyl hydrogen of cholesterol and hydroxyl oxygen on the B-ring. The two hydrogen bonds form a ring-like hydrogen bonding network.

The stable EGC-Chol complexes with energy difference within 5 kcal/mol are also shown in Figure 2. Similar to the EC-Chol complexes, hydrogen bonds with



	Complexes	Eads (kcal/mol)	H-bond distances (Å)
EC-Chol	EC-iso-1	0.0	1.80
	EC-iso-2	1.7	1.83
	EC-iso-3	1.8	2.12; 1.78
EGC-Chol	EGC-iso-1	0.0	1.81
	EGC-iso-2	1.5	1.85; 2.04
	EGC-iso-3	2.6	2.15; 2.01

Figure 2: Molecular structures, E_{ads} and H-bond distances of EC-cholesterol (EC-Chol) and EGC-cholesterol (EGC-Chol).

a bond length of 1.81 Å are generated in the same way for the most stable structure (EGC-iso-1). The EGC-iso-2 has a relative energy of 1.5 kcal/mol, in which two hydrogen bonds with bond lengths of 1.85 and 2.04 Å are formed. The two hydrogen bonds form a ring-like structure. The complex EGC-iso-3 has a relative energy of 2.6 kcal/mol, in which two hydrogen bonds with bond lengths of 2.01 and 2.15 Å are formed as well. From these stable complexes with different adsorption sites, the molecular interactions between EC/EGC and cholesterol are due to the interactions of hydrogen bonds. However, unlike the intuitive understanding of a complex formed by hydrogen bond interactions, which is positively correlated with the number of hydrogen bonds [37,38], the EC-iso-1 and EGC-iso-1 with only one hydrogen bond are more stable than other isomers with a hydrogen bond network.

The structures of ECG-Chol and EGCG-Chol complexes with energy difference within 5 kcal/mol are shown in Figure S2. For the most stable structures (ECG-iso-1 and EGCG-iso-1), one hydrogen bond is formed between hydroxyl oxygen of cholesterol and hydroxyl hydrogen on the B-ring of ECG or EGCG and another hydrogen bond is formed between carbonyl oxygen of gallic acid ester and hydroxyl hydrogen of cholesterol. For the other isomers of ECG-Chol, the relative energy of the complex ECG-iso-2 is 3.2 kcal/mol, and a length of 1.77 Å hydrogen bond is formed. The ECG-iso-3 has a relative energy of 4.1 kcal/mol, in which two hydrogen bonds with bond lengths of 1.70 and 1.96 Å are formed as a ring-like structure. The energies and hydrogen bonds for isomers of EGCG-Chol are similar to those of ECG-Chol.

To analyze the adsorption sites more clearly, we marked them on a schematic as shown in Figure 3. The adsorption sites of EC-Chol and EGC-Chol are on the

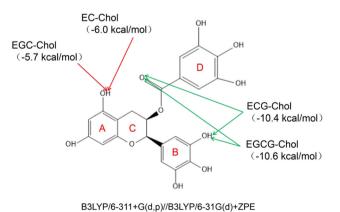


Figure 3: Schematic diagram of adsorption sites.

hydroxyl group of the A-ring, and the adsorption sites of ECG-Chol and EGCG-Chol are on the carbonyl group of gallic acid ester and the hydroxyl group of the B-ring, respectively. Regardless of the hydrogen bonds formed by carbonyl oxygen of gallic acid ester, all the other hydrogen bonds were formed between the hydroxyl hydrogen of catechins and the hydroxyl oxygen of cholesterol.

3.2 Energy of adsorption

To compare the interaction of four catechins on cholesterol, we define the adsorption energy ($E_{\rm ads}$) as

$$E_{\text{ads}} = E_{\text{catechin-cholesterol}} - (E_{\text{catechin}} + E_{\text{cholesterol}}),$$
 (1)

where $E_{\rm catechin}$, $E_{\rm cholesterol}$ and $E_{\rm catechin-cholesterol}$ are the total energies of catechin, cholesterol and complex of catechin-cholesterol, respectively. As shown in Figure 4, $E_{\rm ads}$ of the cholesterol-cholesterol complex is -2.6 kcal/mol, and $E_{\rm ads}$ of the EC-Chol, EGC-Chol, ECG-Chol and EGCG-Chol are -6.0, -5.7, -10.4 and -10.6 kcal/mol, respectively, which are all significantly lower than that of the cholesterol-cholesterol complex, indicating that catechins have an advantage of reducing cholesterol micelle formation. Besides, the results of $E_{\rm ads}$ are consistent with the experimental results that EGCG is more effective than ECG in cholesterol deposition in mice and that EC and EGC are equally effective but weaker than their gallate esters [39]. $E_{\rm ads}$ of EC-Chol is similar to that of EGC-Chol, and $E_{\rm ads}$ of the ECG-Chol and

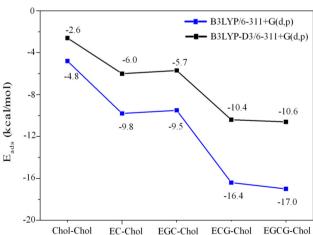


Figure 4: Adsorption energy of complexes (Chol represents cholesterol).

EGCG-Chol complex are similar as well, which is due to the similarity in adsorption sites. In addition, the E_{ads} of ECG-Chol and EGCG-Chol are lower than those of EC-Chol and EGC-Chol, which indicates that the added D-ring containing the gallate significantly enhances the interaction of catechins with cholesterol. Besides, according to Table S2, the adsorption energies of EC-iso-2 and EC-iso-3 are -4.3 and -4.2 kcal/mol, respectively, both higher than that of the most stable structure (EC-iso-1) at -6.0 kcal/mol. Similarly, the adsorption energies of EGC-Chol-2, EGC-Chol-3, ECG-Chol-2, ECG-Chol-3 and EGCG-Chol-2 are higher than that of their most stable structure, respectively, indicating that the most stable complex has the strongest adsorption energy.

Considering the molecular structures contain saturated and aromatic rings, in which dispersion effects may have significant contributions, we have further performed calculations to observe the intermolecular interactions between cholesterol and catechins with the B3LYP-D3 method to verify the reliability of our results. The adsorption energies of all compounds extrapolated using the B3LYP-D3 method are much stronger than the adsorption energies extrapolated using B3LYP as shown in Figure 4, indicating a significant contribution from dispersion effects. Despite the adsorption energies from the B3LYP-D3 provided more reasonable accuracy for interactions between the cholesterol and catechins than those calculated from B3LYP, the difference in adsorption energies between different catechin complexes obtained from B3LYP-D3 is consistent with the results obtained from B3LYP. For example, the adsorption between four catechins and cholesterol is stronger than the adsorption between cholesterol themselves. From the two methods, it has been found that the EGCG still has the strongest adsorption energy for cholesterol. Thus, the conclusion from the B3LYP method is qualitatively reliable.

3.3 Charge analysis

Considering the hydrogen bond is primarily an electrostatic force of attraction, we analyzed and observed a significant charge transfer between the catechin and the cholesterol, which was characterized by the difference in atomic charge before and after adsorption. The total charge of individual molecules in the complex is shown in Figure 5, and the detailed atomic charges are listed in Table S3. Before adsorption, catechins and cholesterol are electrically neutral. After adsorption, the charge transfers from cholesterol to catechin are 0.043, 0.042,

0.024 and 0.028 e for EC-Chol, EGC-Chol, ECG-Chol and EGCG-Chol, respectively, all of which are greater than 0.013 e of cholesterol transferred to another cholesterol.

Besides the intermolecular charge transfer between the catechin and the cholesterol, obvious intramolecular charge transfer is observed in catechins. ECG-Chol and EGCG-Chol also have intramolecular charge transfer of 0.022 and 0.020 e, respectively. Moreover, the charge of the groups at the adsorption sites increases obviously, such as the charge of hydroxyl group of EC at the adsorption site increases by -0.010e after adsorption. Besides, the change in the C atoms on the A-ring connected to the hydroxyl group at the adsorption site is more obvious, with a change of -0.016 e. Similar results were obtained from the other three catechin complexes, such as the charge of hydroxyl group of EGC-Chol increases by -0.010 e, and the charge of the C atoms on the A-ring changes by -0.016e. For the ECG complex, the charges of hydroxyl group, ester bond, B-ring at the adsorption sites and D-ring change by -0.010, -0.012, -0.032 and 0.022 e, respectively. For the EGCG complex, the corresponding charges change by -0.013, -0.011, -0.030 and 0.020 e, respectively.

According to the aforementioned analysis, the interaction between catechins and cholesterol is mainly caused by hydrogen bonds; however, the intermolecular and intramolecular (between the aromatic ring and the hydroxyl group) charge transfers promoted by catechins greatly enhance the hydrogen bonds, resulting in the interactions between catechins and cholesterol, which are much stronger than the hydrogen bond between cholesterols themselves. Therefore, we speculate that the adsorption of catechin and cholesterol is mainly due to the aromatic rings and hydrogen bond, in which the aromatic rings play a dominant role to enhance the hydrogen bonds.

3.4 Electron density distribution

The molecular orbitals were analyzed to further understand the interactions between catechins and cholesterol. The highest occupied states of the molecular orbitals (HOMO) are displayed in Figure 6. The HOMO of EC and EGC are mainly distributed on C and O of the A-ring as well as a little distribution is located at C and O of the C-ring, which are consistent with the behavior that the charge of the A-ring on the two complexes decreased by 0.016 e. Considering the behavior that the

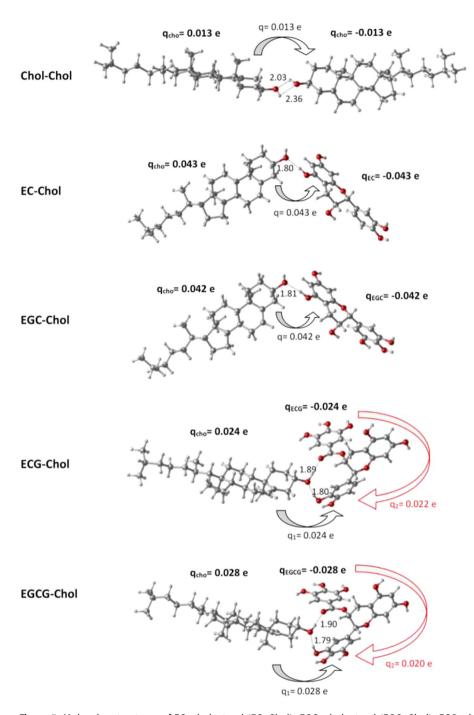


Figure 5: Molecular structures of EC-cholesterol (EC-Chol), EGC-cholesterol (EGC-Chol), ECG-cholesterol (ECG-Chol), EGCG-cholesterol (EGCG-Chol) and cholesterol-cholesterol (Chol-Chol) (the black arrows represent intermolecular charge transfer and the red arrows represent intramolecular charge transfer).

charge of the B-ring on the ECG-Chol and EGCG-Chol complexes decreased by 0.030-0.032 *e*, the HOMO of ECG and EGCG are mainly located on the C and O of the B-ring and partial distribution located at the C and O of A-ring and C-ring.

From the distribution of the aforementioned HOMO, we draw a conclusion that the chemical activities of EC

and EGC are mainly concentrated in the A-ring and those of ECG and EGCG are mainly concentrated in the B-ring. For the catechin–cholesterol complexes, there is a partial electron transfer from the delocalized π orbitals of the aromatic ring to the adjacent hydroxyl, resulting in the most stable complexes with high adsorption energy at these sites. The results are consistent with the

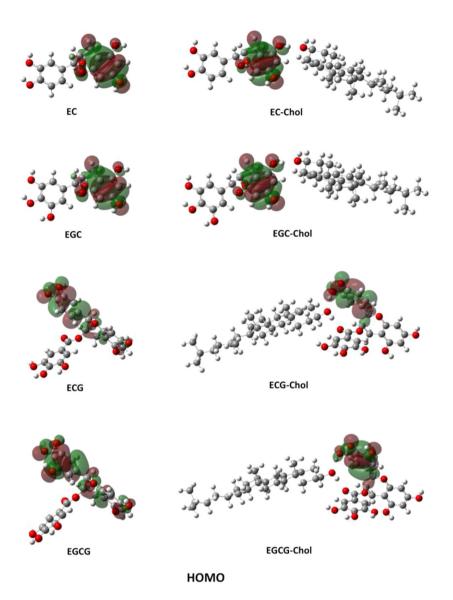


Figure 6: HOMO of EC, EGC, EGCG and catechin complexes at the level of B3LYP/6-31G(d).

adsorption site of the most stable catechin-cholesterol complexes obtained previously, indicating that the aromatic ring plays a dominant role and enhances the adjacent hydrogen bonding through charge transfer.

3.5 PDOS

Statistical analyses of PDOS were performed to analyze the interaction mechanisms. The PDOS of the four catechins, cholesterol and catechin-cholesterol complexes are shown in Figures S3 and S4. The energy gaps of EC, EGC, ECG and EGCG are narrower than that of cholesterol as well as the narrower energy gaps of complexes, which indicates that the chemical activity

of catechin is higher than that of cholesterol during adsorption. The HOMO of catechins are mainly composed of C atoms, while the HOMO of complexes are mainly composed of C atoms as well. The narrower energy gaps and PDOS of HOMO show strong interaction of catechins in which C atoms on aromatic rings play a dominant role.

To clearly show the interaction between cholesterol and catechin, the PDOS of some atoms at the active site before and after adsorption (corresponding to groups in charge transfer statistics) was analyzed, as shown in Figure 7. It can be noted that the distribution of the PDOS of the atoms at the action sites on cholesterol and catechins changed significantly compared with those before adsorption. The PDOS of the electron in the hydroxyl group on cholesterols reduce and shift to low

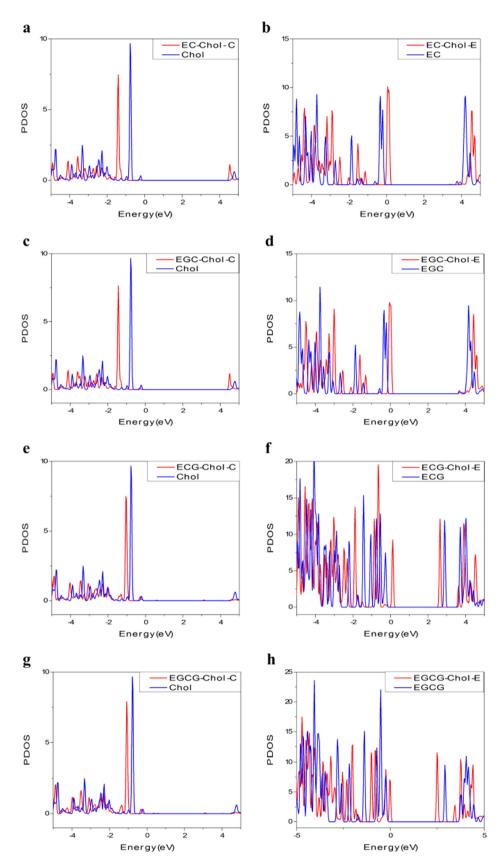


Figure 7: Partial density of the interacting atoms on complexes (catechin–Chol-E represents the active atoms on catechin of catechin–Chol complexes, catechin–Chol-C represents the active atoms on cholesterol of catechin–Chol complexes, Chol represents the active atoms on only cholesterol and EC/EGC/ECG/EGCG represents the active atoms on only catechin).

energy after adsorption as compared to those before adsorption (Figure 7a, c, e, and g), and the PDOS of the electron in the atoms of adsorption sites on catechins increase and shift to high energy after adsorption as compared to those before adsorption (Figure 7b, d, f, and h). The changes in the PDOS of electrons of the cholesterol and catechins as compared to those before adsorption show the corresponding charge transfer between the cholesterol and the catechins. The result is consistent with the charge transfer and charge data described earlier.

In short, the HOMO of the four catechins and their complexes are mainly determined by aromatic rings. Catechins are more active and play a major role in the adsorption process. Moreover, the changes in the PDOS of the active atoms indicate the phenomenon of charge transfer between them as well as the direction of transfer. This result is in good agreement with our

results of adsorption structures, adsorption energy and charge transfer, which proves that the molecular interactions of the complexes are mainly due to charge transfer of the aromatic rings of the catechins as well as the interactions of the hydrogen bonds between catechins and cholesterol.

3.6 Infrared (IR) spectra

We calculated the IR spectra of four catechins, cholesterol and four catechin–cholesterol complexes at the B3LYP/6-31G (d) level, as shown in Figure 8. Upon comparing the IR spectra of EC and cholesterol, it is found that a new peak appeared at 3498.5 cm⁻¹ for the EC–Chol complex, which is caused by a new stretching vibration of the hydroxyl group at the adsorption site on EC. The yellow shading in Figure 8

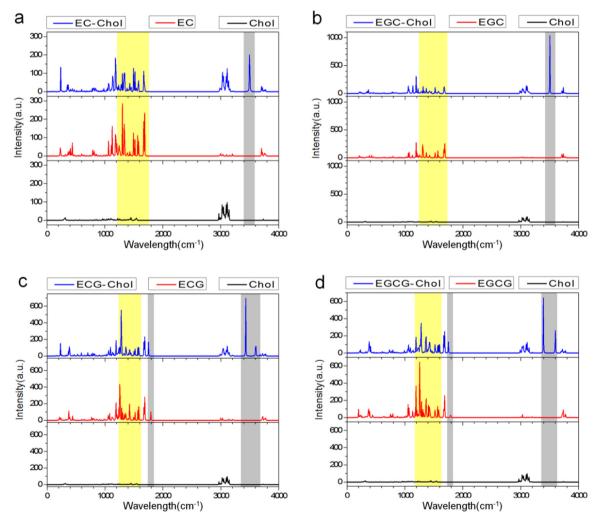


Figure 8: IR spectra of catechin, cholesterol and catechin-cholesterol complex.

corresponds to the characteristic peak of the A-ring at 1669.1 cm⁻¹, which weakens after adsorption. The intensity of the 1278.0 cm⁻¹ peak corresponding to the rocking vibration of the A-ring decreases after adsorption. As shown in Figure 8b, the IR spectrum of the EGC–Chol complex shows a new peak at 3502.3 cm⁻¹, which could be attributed to the hydrogen–oxygen stretching vibration of the hydroxyl group at the adsorption site on EGC. The intensities of 1278.5 and 1668.34 cm⁻¹ generated by A-ring vibration weaken similarly after adsorption.

Similarly, for ECG-Chol in Figure 8c, new peaks appeared at 1745.7, 3429.7 and 3603.2 cm⁻¹. It could be known that the peak at 1745.7 cm⁻¹ is caused by the blue shift of the stretching vibration of carbonyl carbon and oxygen on the gallate in the adsorption site on ECG. The peak at 3429.7 cm⁻¹ is attributed to the stretching vibration of the hydroxyl group in the adsorption site on ECG, while the peak at 3603.2 cm⁻¹ is assigned to the stretching vibration of the hydroxyl group in the adsorption site on cholesterol. The intensity of peaks at 1421.8 and 1496.1 cm⁻¹ weakens after adsorption, which is attributed to the rocking vibration of the D-ring and Bring, respectively. In Figure 8d, the IR spectra of the EGCG-Chol complex show new peaks at 1745.2, 3392.1 and $3596.9 \,\mathrm{cm}^{-1}$. The peak at $1745.2 \,\mathrm{cm}^{-1}$ is caused by the blue shift of the stretching vibration of carbon and oxygen of the carbonyl group on gallate in the adsorption site on EGCG. The new peak at 3392.1 cm⁻¹ can be assigned to the hydrogen-oxygen stretching vibration of the hydroxyl group in the adsorption site on EGCG, while the peak at 3596.9 cm⁻¹ is attributed to the hydrogen-oxygen stretching vibration of the hydroxyl group in the adsorption site on cholesterol. After adsorption, the peak intensity at 1271.4 cm⁻¹ generated by the B-ring weakens.

The results suggest that the adsorption of catechin and cholesterol mainly depends on hydrogen bonds and aromatic rings. For example, the main action rings of EC–Chol and EGC–Chol are the A-ring, while the main action rings of ECG–Chol and EGCG–Chol are the B-ring.

4 Conclusion

In this work, the interaction and mechanism of the catechins and cholesterol have been investigated using the DFT. By simulating the adsorption of catechins and cholesterol, it is found that the adsorption energy produced by catechin on cholesterol is greater than

that produced by cholesterol itself, indicating that catechin has more advantages in reducing the formation of cholesterol micelle, which is consistent with the experimental results [24–27]. Since the molecular structures are similar, EC and EGC have similar adsorption capacity, and the binding sites of the molecular structures with the largest adsorption energy are the same. Similarly, ECG and EGCG have similar adsorption capacity and the binding sites because of their alike molecular structures. Compared with EC and EGC, ECG and EGCG have larger adsorption energy and greater adsorption advantages, which is in line with the result that EGCG is more effective than ECG in cholesterol deposition in mice and that EC and EGC are equally effective but weaker than their gallate esters [39].

In addition, charge, PDOS, electron orbital data and IR spectrum show that the adsorption of cholesterol by EC and EGC mainly depends on the transfer of electrons from the partially delocalized orbitals on the A-ring to enhance the strength of the hydrogen bonds produced. The adsorption of cholesterol by ECG and EGCG depends largely on the transfer of electrons from the partially delocalized orbitals on the B-ring to enhance the strength of the hydrogen bonds generated on aromatic rings, which is accompanied by the effect of the hydrogen bond formed by carbonyl oxygen on the ester bond. It shows the active reaction sites of different kinds of catechins, which help to infer the reaction of catechins with other substances. Besides, unlike intuitive understanding of a complex formed by interaction of hydrogen bonds, which is positively correlated with the number of hydrogen bonds, the strength of hydrogen bond is mainly determined by the distribution of active sites and the intermolecular and intramolecular charge transfer of catechin aromatic rings, thus affecting the stability of the complex.

These aforementioned results suggest a strong possibility that catechins and cholesterol interact through hydrogen bonds. To some extent, it suggests that catechins may influence cholesterol micelles by forming insoluble substances with cholesterol, so as to reduce the absorption of cholesterol by the intestine. This helps us to understand the mechanism by which catechins weaken cholesterol absorption in the intestine by reducing the solubility of cholesterol micelles, so that we can better understand the whole complex mechanism of catechins lowering cholesterol. This can also provide theoretical basis for the development of catechin drugs.

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Conflict of interest: The authors state no conflict of interest.

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