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It was shown that unlike other food acids, the caffeic one exhibits a significant synergistic effect with glutathione in the $ABTS^{+\bullet}$ assay. It was found that the main stages of mechanism of this synergistic interaction is determined by the presence of the 3-OH and 4-OH hydroxyl groups in the structure of caffeic acid and the greater stability of its anion which contains the deprotonated 4-OH hydroxyl group compared with the anion with its deprotonated carboxyl group.

Keywords: *synergistic effect, caffeic acid and glutathione, $ABTS^{+\bullet}$ test, DFT study.*

INTRODUCTION

In general, identifying the mechanisms of synergistic interaction between different pairs of antioxidants is significantly complicated both by the complexity of the antioxidant systems themselves with the corresponding radicals, and by the presence of various aspects associated with the occurrence of a number of simultaneous and coupled reactions in the liquid phase (see, for example, the rewievs [1, 2] and references herein). In this regard, there is practically no data that would allow us to identify the main stages describing synergistic effects in multicomponent systems. Therefore, theoretical studies of the intimate details of the redistribution of electron density between the reagents of the antiradical reactions under study are of particular value. So, for instance, the such theoretical considerations were done for investigation of antioxydant action of different organic acids on the free radicals $DPPH^{\bullet}$ and $ABTS^{+\bullet}$ [3, 4].

The present work is devoted to the DFT study of the synergistic effect between caffeic acid with glutathione (which also is known as a powerful natural antioxidant) in their joint reaction with the $ABTS^{+*}$ cation-radical. The structure of the reduced (and active) form of glutathione (usually denoted as GSH) is shown in Figure 1.

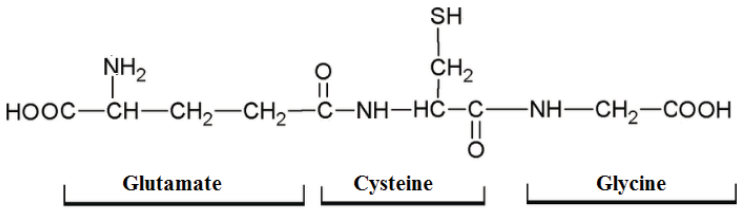


Fig. 1. The structural formula of the reduced form (GSH) of glutathione.

The GSH structure presented in Figure 1 shows that glutathione (gamma-L-glutamyl-L-cysteinylglycine) is the tripeptide synthesized from glutamic acid, cysteine, and glycine.

The experimental revealing of the synergistic effect of various binary mixtures (glutathione + phenolic acid) reacting with the $ABTS^{+*}$ radical cation was studied in Ref. [5]. In the work [5] it was shown that among all the studied food acids, the only caffeic acid exhibits a clear-cut significant synergistic effect. However, the mechanism of this synergistic effect was not determined. The purpose of the present paper is the determination of the main stages of the above synergistic effects mechanism.

COMPUTATIONAL METHOD

Calculations of the geometrical and electronic structures of all the studied molecular systems were performed by the DFT B3LYP method in the basis 6-31G(d) set [6-8]. All calculations were carried out taking into account the solvent (ethanol) based on the IEFPCM option of the Gaussian 09 program package [9].

RESULTS AND DISCUSSION

First of all, our calculations have shown that the most stable anionic form of caffeic acid in ethanol arises by elimination of the protons which belong to the hydroxyl group 4-OH in the structures of the given acids. The main results of our DFT study of the mechanism of the antioxidant synergistic effect between glutathione and caffeic acid in their joint reaction with the $ABTS^{+*}$ radical-cation are presented in the Figures below.

Figure 2 demonstrates the distribution of the electronic density of the unpaired electrons in two complexes of caffeic acid and its anion with the cation-radical $ABTS^{+\bullet}$.

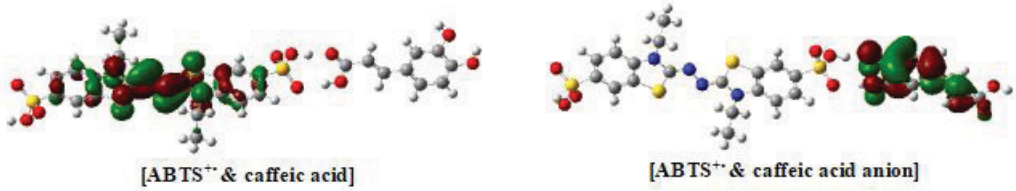


Fig. 2. Spin density distrubutions in the complexes between $ABTS^{+\bullet}$ and caffeic acid (at the top) and $ABTS^{+\bullet}$ with caffeic acid anion (at the bottom).

The first complex [$ABTS^{+\bullet}$ & caffeic acid] at the top of Figure 2 is formed due to the interaction between one sulfo-group of $ABTS^{+*}$ and the carboxyl group of a neutral caffeic acid molecule; the second complex in Figure 2, [$ABTS^{+\bullet}$ & caffeic acid anion], is generated by the interaction of $ABTS^{+\bullet}$ with the most stable caffeic acid anion arising by deprotonation of the para-hydroxyl group of this acid. One can see that the $ABTS^{+*}$ cation radical is transformed into its diamagnetic derivative only in the second case due to the electron density transfer from the anion to $ABTS^{+*}$. However, it should be noted, that in the ethanol medium (as in the aqueous one) only some insignificant part of molecules of caffeic acid is dissociated. Thus, the greater part of the $ABTS^{+*}$ radical-cations reacts with non-dissociated molecules of caffeic acid and, therefore, remains unchanged, i.e. paramagnetic (see the top of Figure 2).

Figure 3 shows the DFT optimized structures of glutathione and its anion.

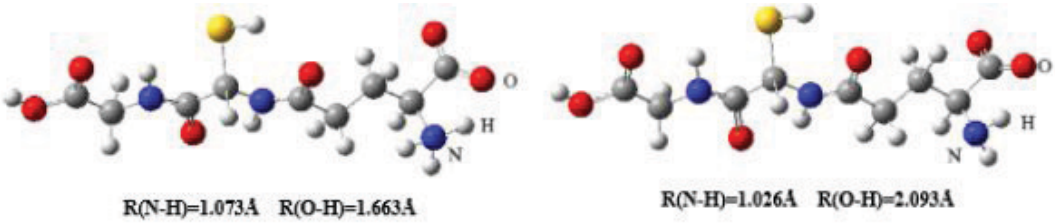


Fig. 3. The optimized geometry structures of the neutral glutathione molecule (left) and its anion (right).

One can see that the neutral glutathione molecule exists in the zwitter-ionic form which contains the $–NH_3^+$ and $–COO^-$ groups. In the glutathione anionic structure the above $–NH_3^+$ group is being transformed into the corresponding amino-group $–NH_2$, which forms the intra-molecular hydrogen bond with the above-considered $–COO^-$ group.

The following DFT calculations show that the glutathione anion can interact with the complex [$ABTS^{+\bullet}$ & caffeic acid] generating the new complex [$ABTS^{+\bullet}$ & caffeic acid & glutathione anion] whose electronic distrubution is shown in Figure 4.

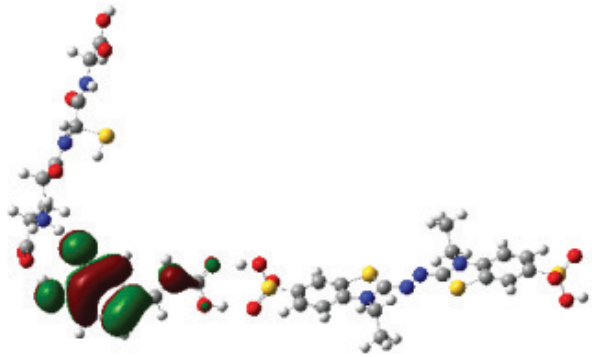


Fig. 4. Unpaired electron density distribution in the common complex [$ABTS^{+\bullet}$ & caffeic acid & glutathione anion].

Thus, all of the above results make it possible to understand the influence of glutathione on the inactive complex [$ABTS^{+\bullet}$ & caffeic acid].

In its turn, caffeic acid enhances antioxidant activity of glutathione interacting with $ABTS^{+*}$. So, Figure 5 shows that the interaction of a neutral molecule of glutathione with the cation-radical $ABTS^{+*}$ does not lead to any electron density transfer from glutathione to the given cation-radical. It means that this interaction does not transform the latter into its diamagnetic derivative.

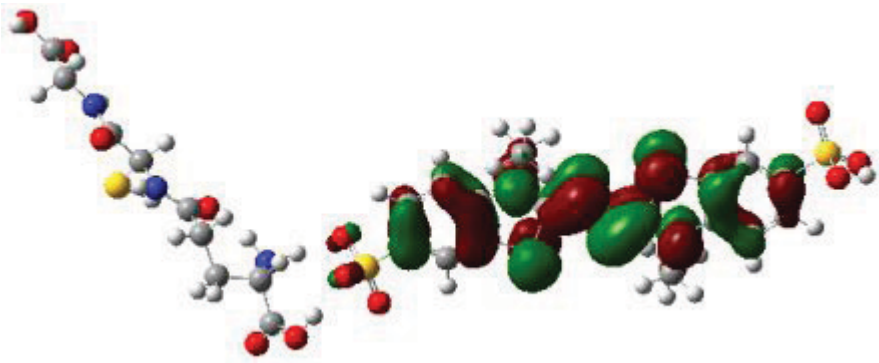


Fig. 5. Unpaired electron density distribution in the complex [$ABTS^{+\bullet}$ & glutathione].

Our further DFT calculations have shown that the neutral moleule of caffeic acid is able to make the relocation of the unpaired electronic density in the complex [$ABTS^{+\bullet}$ & glutathione anion], forming the joint complex [$ABTS^{+\bullet}$ & glutathione anion & caffeic acid] (Figure 6).

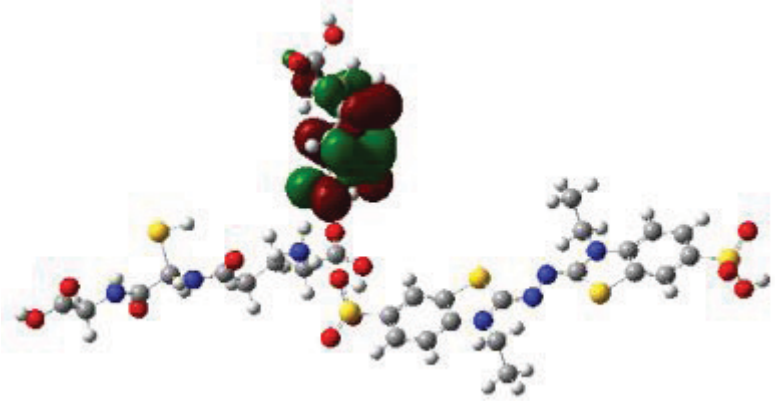


Fig. 6. Unpaired electron density localization in the joint complex [$ABTS^{+\bullet}$ & glutathione anion & caffeic acid].

Practically the same complex is being formed in the case when the anion of caffeic acid reacts with the complex [$ABTS^{+\bullet}$ & glutathione], forming the complex [$ABTS^{+\bullet}$ &

glutathione & caffeic acid anion], whose structure coincides with thjat presented in Figure 6. Thus, the above-considered results of our DFT calculations show that caffeic acid in its both the neutral and anionic forms enhances the antioxidant activity of glutathione, transforming the cation-radical $ABTS^{+*}$ in the complexes [glutathione anion & $ABTS^{+*}$] and [glutathione & $ABTS^{+*}$] into its diamagnetic derivative. The above theoretical results are in full agreement with the experimental data presented in the work [5].

CONCLUSIONS

Thus, our theoretical investigation shows that for the join reaction of caffeic acid and glutathione with the cation-radical $ABTS^{+*}$ the mutual enhancing of the antiradical activities of both these antioxidant compounds takes place ; i.e. the synergistic effect between them is occurs. This effect is realized solely due to the presence of 3-OH and 4-OH hydroxyl groups in the structure of caffeic acid and the formation of the caffeic acid anion due to the deprotonation of the 4-OH hydroxyl group. The present study also provides insight into why other phenolic dietary acids, whose structures do not contain such hydroxyl groups, do not exhibit a synergistic effect with glutathione.

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