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Insights into the inhibition of xanthine oxidase by curcumin

Liang Shen, Hong-Fang Ji *

Shandong Provincial Research Center for Bioinformatic Engineering and Technique, Center for Advanced Study, Shandong University of Technology, Zibo 255049, PR China

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ABSTRACT

As a natural pigment, curcumin exhibits multiple biological activities. Previous studies have investigated the inhibition of xanthine oxidase (XO) by curcumin. In the present work, based on the molecular docking simulations, it is interesting to find that parent curcumin binds weakly to XO, while its degradation products, for example, *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal, exhibit effective inhibitory activities against XO. The findings shed new light on the underlying mechanisms of curcumin in inhibiting XO and also have potential implication that both parent curcumin and its degradation products should be taken into account when exploring the mechanisms of curcumin's biological activities.

Curcumin is a natural yellow pigment, which has attracted much attention in recent years owing to its wide spectrum of biological activities, including anti-oxidant, anti-tumour, anti-inflammatory, and anti-microbial activities, etc.[1–4] Xanthine oxidase (XO), an enzyme capable of generating reactive oxygen species,[5] has been found to play important roles in many pathological conditions and also be involved in the pathogenesis of many diseases.[6] Previous studies have investigated the inhibition of XO by curcumin.[7,8] Lin and Shih reported that curcumin can inhibit XO.[7] In contrast, a recent work by Pauff et al. found that curcumin exhibits no inhibitory activity against XO.[8] Thus, more effort is necessary to elucidate the actions of curcumin in inhibiting XO. By means of molecular docking simulations, it is interesting to find that parent curcumin binds weakly to XO, while its degradation products, such as *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal, ferulic aldehyde, ferulic acid, feruloyl methane and vanillin (Fig. 1),[9] show inhibitory activities against XO. The findings provide some deeper insights into the mechanisms of curcumin in inhibiting XO.

The crystal structure of bovine XO in complex with its competitive inhibitor salicylate was obtained from the protein data bank (PDB ID code 1FIQ[10]). Due to the presence of the molybdopterin cofactor that is responsible of oxidation, the docking study was carried out only on the C subunit of the protein. Firstly, the A- and B-chains of the protein and all small molecules were removed except for the molybdopterin cofactor. Then, all bonds were modified automatically and missing hydrogen atoms were added using Builder Module in Insight II.[11] The partial atomic charges were assigned to XO using CVFF force field[12–14] using Discover Module in Insight II. The binding sites of XO were constructed by using salicylate as reference ligand. Standard parameters of the programme FlexX,[15] as implemented in the molecular modelling software SYBYL 7.0,[16] were used to explore the probable binding interactions of the inhibitors with XO. The Ludi module of Insight II[11] was employed to estimate the binding affinities. The Ludi score derived by the programme is empirically related to the dissociation constant K_d: Ludi score = $-100 \log K_d$.

To verify the present method, the docking calculations have been performed on two polyphenols, quercetin and luteolin, which have been proved to possess high inhibitory activities against XO.[8] The binding modes of quercetin and luteolin in the active site of XO with all interacting residues are depicted in Figure 2. It can be seen that the binding pocket for quercetin to XO consists of six residues, that is, Arg880, Arg912, Phe914, Phe1009, Thr1010 and Glu1261 (Fig. 2a). As to luteolin, six residues, that is, Asn768, Arg880, Phe914, Phe1009, Thr1010 and Ala1079 are involved in the interactions with XO (Fig. 2b). It can be seen that four binding residues are same among the two groups, suggesting that quercetin and luteolin share a common binding region. To estimate the binding affinity of two inhibitors to XO, the Ludi scores for the XO/quercetin and XO/luteolin complexes were calculated, which is 595 for the former and 584 for the later. Then, according to the equation: Ludi score = $-100 \log K_d$, the binding affinity is estimated to be 1.12 μM and 1.45 μM for quercetin and luteolin with XO, respectively. The theoretically estimated K_d are very close to the experimental values (Table 1),[8] which verifies the effectiveness of the present methodology in dealing with these systems.

* Corresponding author. Tel./fax: +86 533 278 0271.
E-mail address: jhf@sdut.edu.cn (H.-F. Ji).

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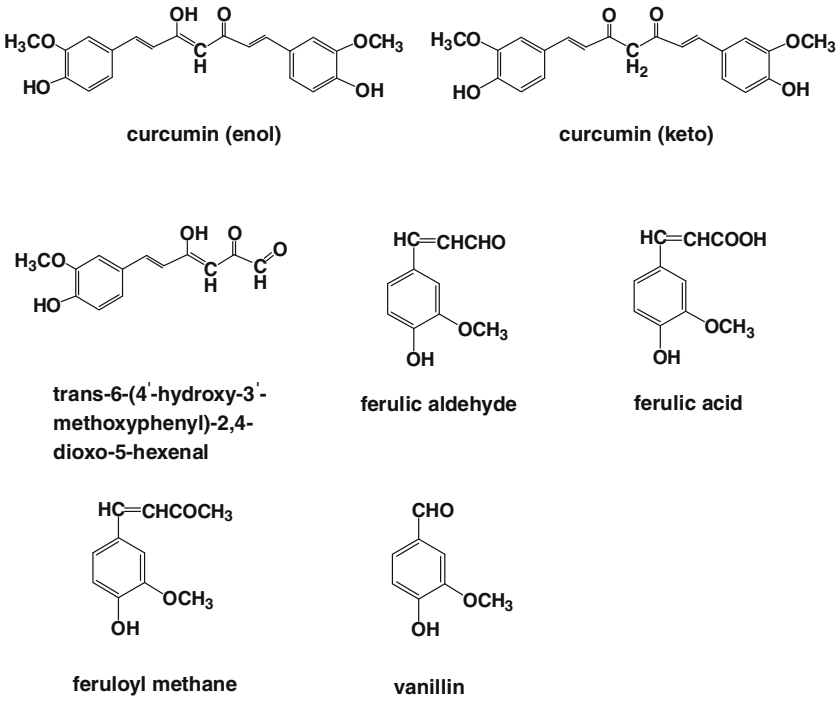

Figure 1. Chemical structures of curcumin and its degradation products.

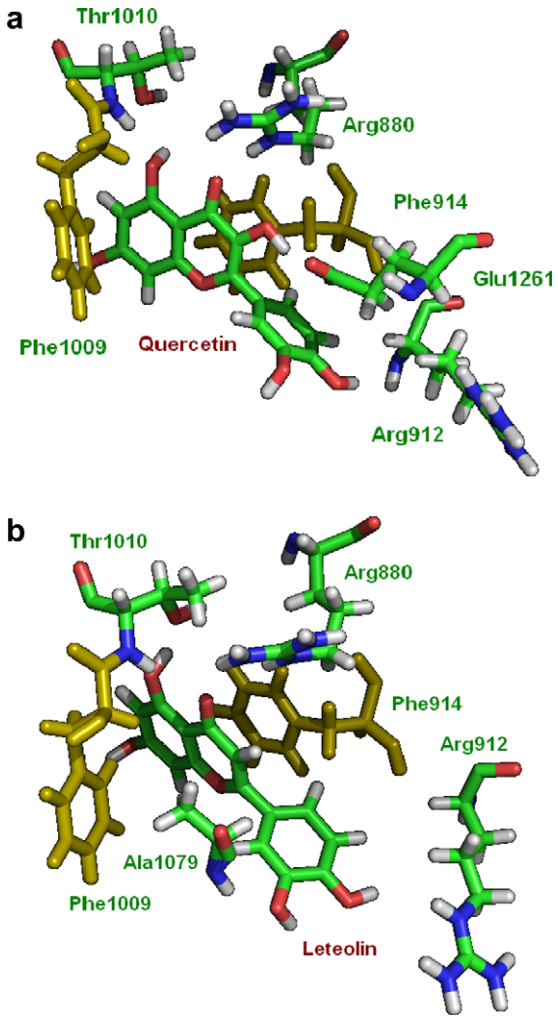

Figure 2. Close-up views of binding modes of XO with quercetin (a) and luteolin (b). The hydrophobic residues are labelled in olive.

Table 1
Theoretically estimated Ludi scores and K_d of the inhibitors with XO

Inhibitors	Ludi score	K_d (μM)	K_i (μM)
Quercetin	595	1.12	1.2 ± 0.7[a]
Luteolin	584	1.45	1.9 ± 0.7[a]
Curcumin (keto form)	385	141	—
Curcumin (enol form)	373	186	—
Trans-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal	534	4.57	—
Ferulic aldehyde	462	24.0	—
Ferulic acid	415	70.8	93.88 ± 18.95[b]
Feruloyl methane	404	91.2	—
Vanillin	401	97.7	—

[a] Experimental value from Ref. 8.
[b] Experimental value from Ref. 17.

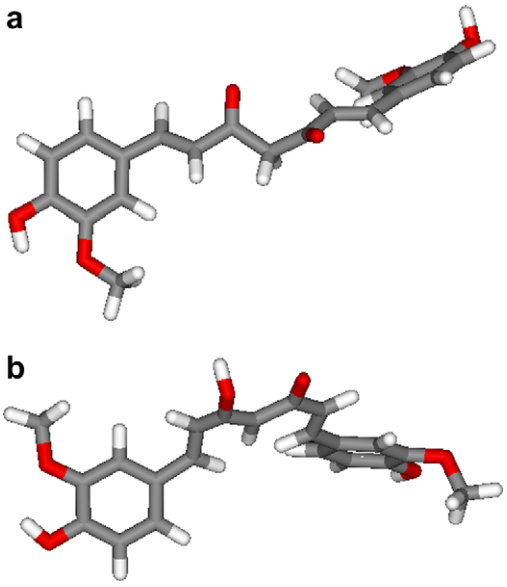

Figure 3. Schematic twisted conformation of keto form (a) and enol form (b) of curcumin in XO.

Then, curcumin was also docked to XO using the same methods. However, both the keto and enol forms of curcumin fail to fit the binding pocket efficiently and can only enter the binding cleft. This mainly arises from the twisted steric bulk geometry of curcumin (Fig. 3), which hindered it to access the binding site completely. The low inhibitory efficiency is also reflected by the estimated binding affinities of the complexes, which is 141 μM and 186 μM for the keto and enol forms of curcumin with XO, respectively.

As it has been reported that curcumin is unstable under physiological condition and *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal was identified as major degradation product, and ferulic aldehyde, ferulic acid, feruloyl methane and vanillin were identified as minor degradation products.[9] Thus, it is reasonable to speculate that these degradation products may be responsible for the experimentally observed inhibition of curcumin against XO.[7] Therefore, parallel docking simulations have also been performed on the degradation products of curcumin with XO and the theoretical binding sites are shown in Figure 4. Comparing with parent curcumin which consists of two feruloyl moieties connected by the methylene bridge, the five degradation products retain only one feruloyl part. Without the steric effects, they all can enter into the cavity successfully. Given the several same residues (Phe914, Phe1009 and Thr1010) involved in the interactions of the five compounds with XO according to the binding modes, the general

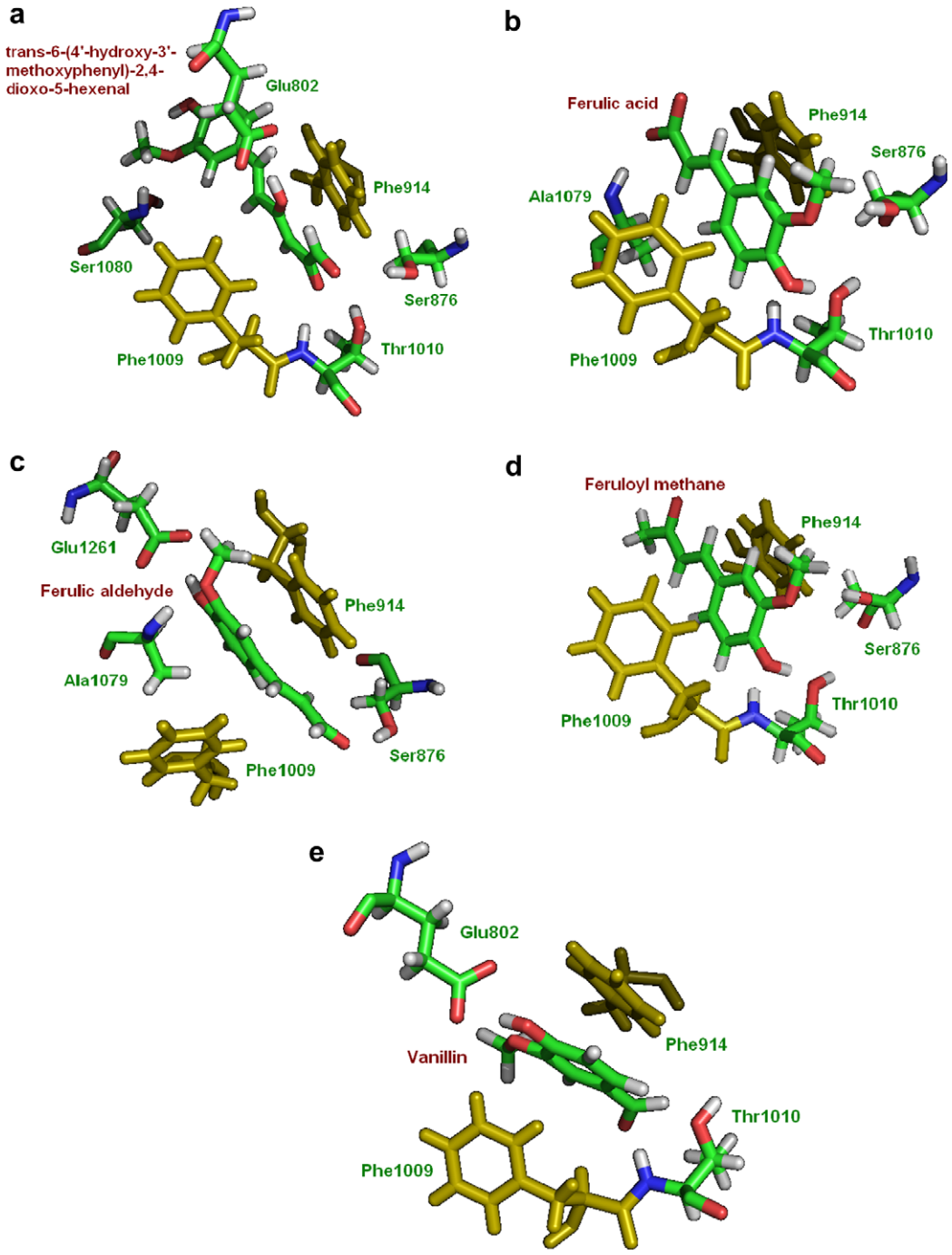

Figure 4. Close-up views of binding modes of XO with *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal (a), ferulic acid (b), ferulic aldehyde (c), feruloyl methane (d) and vanillin (e). The hydrophobic residues are labelled in olive.

locations of their binding sites are similar to each other and also to those of quercetin and luteolin (Figs. 2 and 4).

The binding constants of the five degradation products with XO are estimated and listed in Table 1. Among the five inhibitors, the major degradation product, *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal, possesses comparable activity to those of quercetin and luteolin (Table 1). According to the theoretical K_d, the inhibitory activities of the four minor degradation products are lower than that of *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal. The theoretical result is also close to the experimental value. For instance, the theoretical K_d of ferulic acid to XO is 70.8 μM, which is near to the experimental value 93.88 ± 18.95 μM,[17] which further verifies the accuracy of the methods and also ensures the reliance of the theoretically predicted binding affinity. Therefore, according to the present results, the degradation products can inhibit XO and the inhibition may account for the experimentally observed inhibitory activity of curcumin against XO.[7]

In summary, the present docking simulation study suggests that curcumin binds weakly to XO, while its degradation products, especially the major one, *trans*-6-(4′-hydroxy-3′-methoxyphenyl)-2,4-dioxo-5-hexenal, show inhibitory activity against XO. The opposite reports in previous studies on the inhibition of curcumin against XO may arise from the different experimental conditions,[7,8] which accelerated or hindered the degradation of curcumin, and thus, obtained positive or negative results. This provides some deeper insights into the actions of curcumin in inhibiting XO. Moreover, the present findings imply that the degradation products should also be given full attention when elucidating the biological activities of curcumin, which is unstable under physiological conditions.

Acknowledgements

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