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RESEARCH ARTICLE

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Inhibitory effects of curcumin derivatives on nonenzymatic glucosylation *in vitro*

Abstract In this paper, curcumin, demethoxycurcumin, bisdemethoxycurcumin and another curcumin derivative are investigated for their inhibitory effects on nonenzymatic glucosylation in vitro, their binding reaction with bovine serum albumin (BSA) and their influence on the conformational changes of BSA. It demonstrated that all of these curcumin derivatives inhibited the formation of advanced glycation end products (AGE). Curcumin showed the most potent inhibitory activity, followed by demethoxycurcumin and bisdemethoxycurcumin. Moreover, it indicated that they extensively binded to the protein and induced the conformational changes of BSA.

Keywords nonenzymatic glucosylation, advanced glycation end products (AGEs), curcumin derivatives, binding reaction

1 Introduction

The recent diabetes control and complication trial [1] confirmed that patients with higher levels of mean blood glucose have a higher prevalence of diabetic complications. This supported the hypothesis of many investigators [2, 3] that significant pathogenic alterations arose from irreversible nonenzymatic chemical modifications of plasma and tissue components that were initiated by glucose. Glucose initially reacted with amino acids and proteins to form a Schiff base, and then underwent an Amadori rearrangement to form a stable compound called the Amadori product. Further Maillard reactions ensued to slowly produce reactive and toxic covalent adducts of largely unknown structures that were often termed as advanced glycation end products or "AGEs." Therefore, targeting glucosylation should potentially be of much medical and pharmacological importance.

Curcumin (1,7-bis(4-hydroxy-3- methoxyphenyl)-1,6-heptadiene-3,5-dione, shown in Scheme 1) and its analogues were isolated from turmeric (dry rhizomes of *Curcumin longa*), a commonly used spice and food colorant. These compounds had been reported to possess a variety of biological and pharmacological activities, including anti-oxidative, anti-inflammatory, anti-carcinogenic, anti-diabetic and anti-HIV. In this paper, we reported the inhibition of protein glucosylation by curcumin (1) and its analogues, including demethoxycurcumin (2), bisdemethoxycurcumin (3) and curcuminoid a (4) (see Fig. 1).

2 Materials and methods

2.1 Materials

All solvents and other chemicals used were of analytical grade and obtained from Dongzhen Chemical Co. (Guangzhou, China). Bovine serum albumin (BSA) was obtained from Huamei Biological Co. (Guangzhou, China) and D-fructose was obtained from Sigma Chemical Co.

2.2 Synthesis, isolation and purification of curcuminoids

Curcumin, demethoxycurcumin, bisdemethoxycurcumin were isolated from CRTO and identified according to earlier

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studies [4, 5]; its analogue 4 was synthesized.

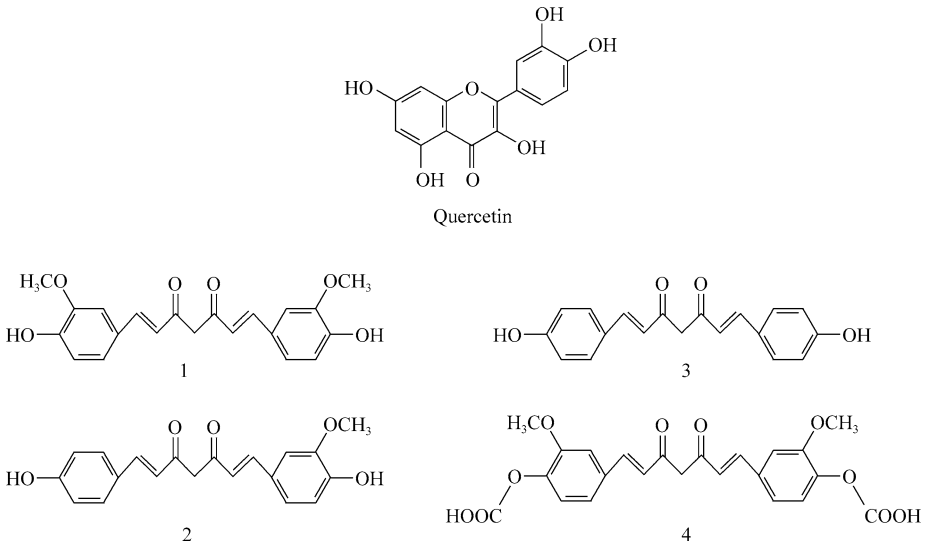


Fig.1 Chemical structures of the compound

2.3 Inhibition of BSA glucosylation

The procedure followed that of Sun et al. [6] and was modified. Bovine serum albumin (BSA; 2 mg/ml) was incubated with D-fructose (200 mM) in carbonate buffer (20 mM, pH 9.0) at 55°C in the presence and absence of the extracts for ten days.

DMSO used for dissolving the residue was found to have no effect on the reaction at <2% (v/v). The concentrations of the extract are as follows: 0, 10, 25, 50, 100 and 150 μM. A concentration of AG and BSA (without the extract) was used as the positive and negative controls, respectively. AGE formations were measured by determining the fluorescence by excitation at 340 nm and emission at 420 nm using an FLS 920 fluorescence spectrometer. Inhibition % was calculated according to the following equation:

(% inhibitory capacity for AGEs formation in BSA)

$$= \left(1 - \frac{fluorescenceofsampleexat\,340nm; em.at\,420nm}{fluorescenceofcontrolex.at\,340nm; em.at\,420nm}\right) \times 100\% \quad (1)$$

IC50 values were obtained from the linear regression plots between the percent inhibitions vs. concentrations.

2.4 Measuring the CD/UV spectra of curcuminoids-BSA complex

The ligand binding assay was carried out by pipetting 2.0 ml of mixture containing 1 mg ml^{-1} BSA and 1.25 mmol/L NaN_3. The concentrations of the extract were 0, 10, 25, 50, 100, 150 μM, respectively. The blank cell was filled with 400 μL concentration of 500 mmol/L instead of the extract. Fluorescence spectra (Ex 282 nm/Em 341 nm) were taken using Jasco RF-5301PC fluorescence spectroscopy, and recording the CD spectra between 190–260 nm. The following equation was used to obtain the binding constant (K) and molar ratio of BSA and the single molecule (n), where θ is the concentration of the extract [7] .

$$\lg\left[\left(F_0 - F\right)/F\right] = \lg K + n\lg\left[\theta\right] \quad (2)$$

3 Results and discussion

3.1 The inhibition of AGEs production by curcuminoids

In the study, quercetin, which was reported to be a high activity inhibitor, was used as reference compound. BSA glucosylation inhibition data were expressed as IC_{50} values (the concentration of inhibitor required to produce 50% inhibition of the test models). The activities of these compounds were then reported as inhibition (%) at certain concentrations (Table 1 and Fig. 2).

Table 1 Inhibition of AGEs by the compounds

Compound	$IC_{50\ (\mu mol/L)}$
1	30.5
2	35.0
3	32.0
4	58.0
Quercetin	32.1

Mao et al. demonstrated that streptozotocin-induced diabetic rats had increased aortic collagen fluorescence indicative of glucosylation and that aminoguanidine or quercetin provided protection against formation of these fluorescent adducts [8]. In the studies described here, the inhibition of quercetin was 66% at the 50 μmol/L dose. Compared to quercetin, curcumin, demethoxycurcumin and bisdemethoxycurcumin exerted more potential inhibitory activities at the same dose, however, the synthetic curcumin

4 had a lower activity than the others. Among the curcuminoids, compound 1 exhibited the highest inhibitory activity with an IC_{50} value of 30.5 μM, followed by compound 3

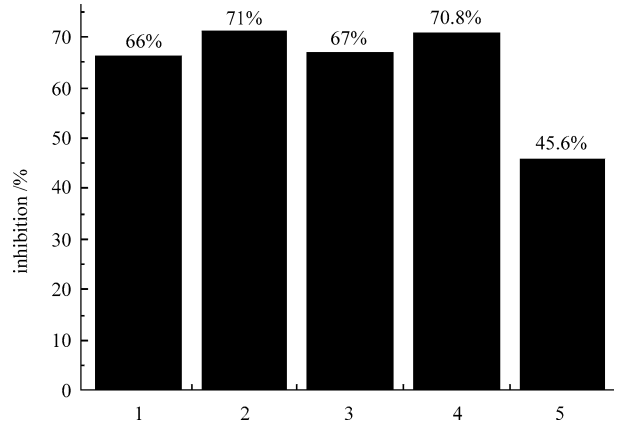


Fig.2 Dose response of % inhibition of gluc osylation protein and AGEs after 10 days of incub ation with different compounds at concentration of 50 μ mol/L($P<0.01$)

*1. Quercetin 2. Curcumin 3. demetho xycurcumin 4. bisde methoxycurcumin 5. curcuminoid a

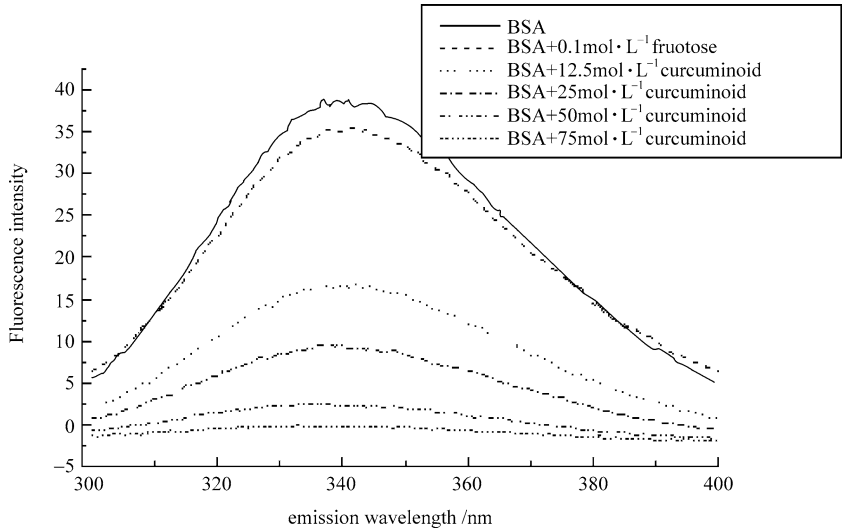
and compound 2 (Table 1). There was no report about the pathway in which curcuminoids inhibited BSA glucosylation.

The result showed that the activity of these compounds depended significantly on the number of phenolic groups in the molecules. Compounds 1, 2 and 3, which had two phenolic groups each, exhibited more activity than compound 4, which had no phenolic group. Symmetry of compound structure was also related to the activity. Compounds 1 and 3 were symmetrical so they were more activated than the others. This might be related to their binding with BSA.

3.2 Binding reaction between curcuminoids and BSA

The emission spectra of BSA in the carbonic acid buffer (pH 9.0) had a maximum of about 300–400 nm at an excitation wavelength of 282 nm (Fig. 3). It was also observed that 0.1 mol/L fructose exerted nearly no influence on the BSA's absorption spectra (Fig 4). In contrast, curcuminoids caused peak intensity reduction, with intensity depending on curcuminoid concentration. When the concentration was lower, the peak intensity reduced faster.

Fig.3 Fluorescence emission spectra of bovine serum albumin(BSA)with scaled in arbttrary units as a function of compounds' concentration



These results indicate that curcuminoids bind more easily to BSA than fructose. In the reaction mixture of fructose, curcuminoids and BSA, cucuminoids interfered with BSA's reaction with fructose by binding to the protein first, thereby inhibiting BSA glucosylation.

We then studied the relationship between protein binding reaction and the inhibition of the BSA glucosylation. We determined the binding constants and the number of binding sites (Table 2) by the slope of the line shown in Fig. 4. Data in Table 2 showed that the binding reaction between curcuminoids and BSA was strong, and that the ratio of the compound to BSA was 2:1.

By comparing the IC_{50} values (Table 1) of compounds 2, 3 and 4, we can determine how inhibitory activity is related to the binding constants and the number of binding sites. Compound 3 had the highest inhibitory activity, binding constant, as well as number of binding sites. This indicated that the mechanism of inhibition was based on the binding reaction between the compound and BSA. The compounds held the sites of BSA, which in turn, inhibited the reaction of fructose and BSA.

Fig.4 Plots of lg[$(F_0$-$F)/F$]vs.1g[θ]

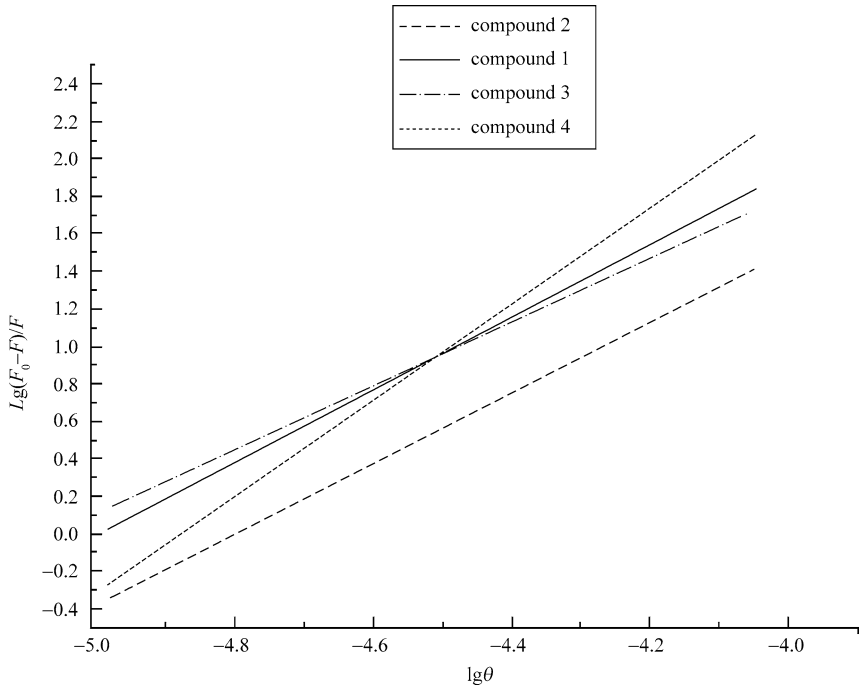


Table 2 The binding constants and the binding site of bovine (BSA) and the compounds

Compounds	Binding constants K*（L • mol^{-1}）	Number of binding site (n)	Correlation coefficient
1	4.65×10^{18}	1.71287	0.99418
2	5.01×10^{9}	1.94242	0.99084
3	3.47×10^{12}	2.57107	0.99863
4	1.06×10^{9}	1.88137	0.9979

* binding degree of BSA with the compounds

Compound 1 was different from the other three in that the inhibitory activity was the strongest as the binding reaction was weak. This suggests that it inhibits BSA glucosylation via a pathway other than the binding reaction. A previous study showed the early stage of the nonenzyme glucosylation producing hydroperoxides and free radicals caused the protein in vivo and played an important role in AGE formation. Curcumin had also been shown to have antioxidant activity in vitro [10] as well as improve the capacity of bearing anoxic in cardiac muscle [11]. We therefore inferred that curcumin inhibited AGE formation by clearing free radical production in the Maillard reaction.

3.3 The influence of curcuminoids on the conformational changes of BSA

According to Fig. 5, BSA solution without the extracts had a negative peak at 243 nm. As the concentration was increased, the negative peak decreased. This indicates that the conformational change of BSA was caused by the addition of the compounds, and that the change was related to the latter's concentration. Of the four compounds, the influence of curcuminoid a was the strongest.

4 Conclusions

Curcumin derivatives could inhibit the production of nonenzymatic glucosylation AGEs effectively in vitro. Curcumin showed the most potent inhibitory activity, followed by demethoxycurcumin, bisdemethoxycurcumin and curcumin derivative a. IC_{50} values were detected below 35 μmol/L on curcumin derivatives which contained hydroxyl. Their inhibitory efficiency was equal to the control sample. It was further proven, via fluoroscopy and circular dichroism spectra, that the inhibitory effect of curcumin derivates on AGEs was caused by the interaction. Based on the mechanism of glucosylation and the antioxidant activity of the curcuminoids, we concluded that the inhibition mechanism was related to their antioxidant capacity.

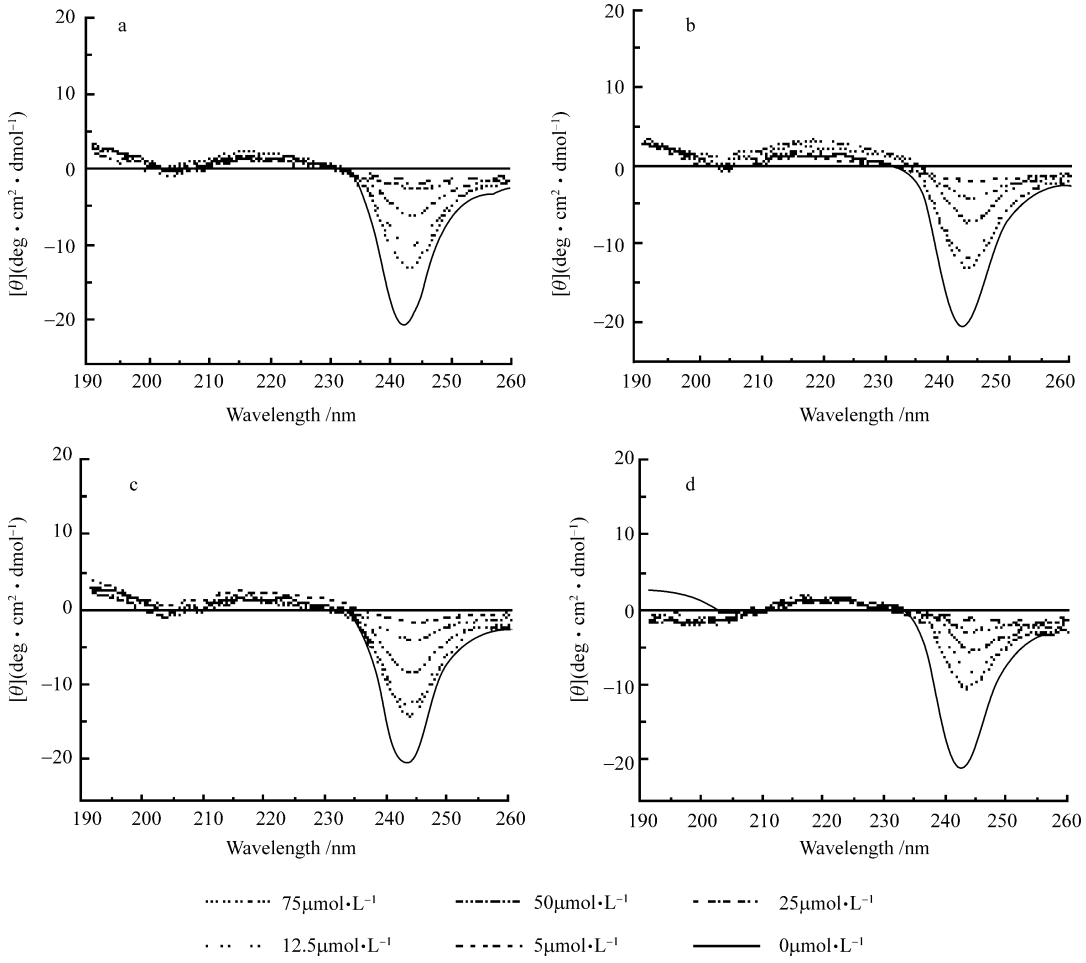


Fig.5 Circular dichuoism spectra of bovine serum albumin(BSA)as a function of compound concentration

References

1. Hongw K. and Spornm B., Recent advances in chemoprevention of cancer, Science, 1997, 278: 1073–1077
2. Metz T. O., Alderson N. L. and Thorpe S. R., Pyridoxamine, an inhibitor of advanced glycation and lipoxidation reactions: a novel therapy for treatment of diabetic complications, Arch. Biochem. Biophys., 2003, 419: 41–49
3. Bierhaus A., Hofmann M. A. and Ziegler R., AGEs and their interaction with AGE-receptors in vascular disease and diabetes mellitus. I. The AGE concept, Cardiovasc. Res., 1998, 37: 586–600
4. Park S. Y. and Kim D. S., Discovery of natural products from C*urcuma longa tha* protects cells from beta-amyloid inset: a drug discovery effort against Alzheimer's disease, J. Nat. Pro., 2002, 65(9): 1227–1231
5. Kim J. H. and Kwon H. J., Novel curcumin derivatives. WO03105751
6. Sun Y. X., Hayakawa S. and Izuemori K., Modification of ovalbumin with a rare ketohexose through the Millard Reaction: effect on protein structure and gel properties, J. Agric. Food Chem., 2004, 52: 1293–1299
7. Feng X., Bai C., Lin Z. et al., The interaction between acridine orange and bovine serum albumin, Chin. J. Anal. Chem., 1998, 26(2): 154
8. Mao P., Zhang J. and Huang Q., Blocking effect of quercetin on the nonenzymatic glucosylation of aortic collagen, Chin. J. Diabetes, 1998, 6(1): 34–37
9. Sua´Rezl G. and Wang X. H., Differential inhibition of maillard protein fluorescence by nitric oxide donors, J. Biol. Chem., 1998, 2(6): 475–480
10. Sharma O. P., Antioxidant activity of curcumin and related compounds, Biochem. Pharmacol., 1976, 25: 1181–1812
11. Shahed A. R., Jones E. and Shokes D., Quercentin and curcumin up-regulate antioxidant gene expression in rat kidney after urethral obstruction or ischemia reperfusion injury, Transplant Proc., 2001, 33(6): 2988