Interaction of Caffeic Acid with Bovine Serum Albumin is Complex: Calorimetric, Spectroscopic and Molecular Docking Evidence

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Fig. S1 ITC binding isotherm displaying the raw data for dilution effect for (A) BSA and (B) CA at 298 K in 0.1 M phosphate buffer (pH 7.4).
Fig. S2 ITC profile for BSA titration with CA. The solid line represents the non-linear least-squares fit to the experimental data points using OneSites Model/TwoSites Model.

Model: OneSites
Reduced $\chi^2 = 3121$
$N = 9.61 \times 10^6 \pm 0.611$ Sites
$K = 8.73 \times 10^3 \pm 1.62 \times 10^3$ M$^{-1}$
$\Delta H = -4.376 \times 10^9 \pm 2.779 \times 10^{14}$ J mol$^{-1}$
$\Delta S = -1.47 \times 10^7$ J mol$^{-1}$ K$^{-1}$

Model: TwoSites
Reduced $\chi^2 = 471.5$
$N_1 = 1.16 \pm 0.528$ Sites
$K_1 = 2.99 \times 10^5 \pm 6.10 \times 10^5$ M$^{-1}$
$\Delta H_1 = -1.40 \times 10^4 \pm 1.01 \times 10^4$ J mol$^{-1}$
$\Delta S_1 = 58.0$ J mol$^{-1}$ K$^{-1}$
$N_2 = 2.84 \pm 2.95$ Sites
$K_2 = 5.55 \times 10^3 \pm 1.93 \times 10^3$ M$^{-1}$
$\Delta H_2 = -9323 \pm 1.11 \times 10^4$ J mol$^{-1}$
$\Delta S_2 = 40.4$ J mol$^{-1}$ K$^{-1}$
Fig. S3 A representative ITC profile displaying the raw data for the integrated heat change (after appropriate correction for heat of dilution) for WAR \((1.5 \times 10^{-3} \text{ M})\) with BSA \((5 \times 10^{-5} \text{ M})\) interaction at 298 K in 0.1 M phosphate buffer pH 7.4. The solid line represents the non-linear least-squares fit to the experimental data points using OneSites Model/Two Sites sequential binding Model.
**Fig. S4** A representative ITC profile displaying the raw data for the integrated heat change (after appropriate correction for heat of dilution) for BSA (5 × 10⁻⁵ M) with IBP (7.75 × 10⁻⁴ M) interaction at 298 K in 0.1 M phosphate buffer pH 7.4.

Model: OneSites

Reduced $\chi^2 = 1.289E5$

$N = 0.68 \pm 0.028$ Sites

$K = 1.44\times10^6 \pm 0.69\times10^6$ M⁻¹

$\Delta H = -2.85\times10^4 \pm 2.56\times10^3$ J mol⁻¹

$\Delta S = 22.30$ J mol⁻¹ K⁻¹
Fig. S5. PeakFit decomposition of DSC thermogram for BSA thermal denaturation. Albumin concentration is $1.05 \times 10^{-4}$ M, scan rate of 1 K/min. The raw data is represented by solid line, PeakFit component 1 by dotted line and PeakFit component 2 by dashed line.
Fig. S6 PeakFit decomposition of DSC thermogram of CA: BSA thermal denaturation at different molar ratio (A) 1:1, (B) 3.5:1, (C) 20:1. Albumin concentration is $1.05 \times 10^{-4}$ M, scan rate of 1 K/min. The raw data is represented by solid line, PeakFit component 1 by dotted line and PeakFit component 2 by dashed line.
Fig. S7 Influence of different concentrations of WAR on BSA thermal denaturation: (A) WAR: BSA 0, (B) WAR: BSA 1:1, (C) WAR: BSA 3:1 molar ratios.

Fig. S8 Influence of competitive binding of WAR and CA on BSA thermal denaturation: (A) WAR: BSA 1:1, (B) WAR: BSA 1:1 and CA $3.70 \times 10^{-4}$ M, (C) BSA and CA $3.70 \times 10^{-4}$ M.
Fig. S9 Influence of competitive binding of WAR and CA on BSA thermal denaturation: (A) WAR: BSA 1:1, (B) WAR: BSA 1:1 and CA $2.10 \times 10^{-3}$ M, (C) BSA and CA $2.10 \times 10^{-3}$ M.

Fig. S10 Influence of competitive binding of WAR and CA on BSA thermal denaturation: (A) BSA, (B) WAR: BSA 3:1, (C) WAR: BSA 3:1 and CA $3.70 \times 10^{-4}$ M.
**Fig. S11** Influence of different concentrations of IBP on BSA thermal denaturation: (A) IBP: BSA 0, (B) IBP: BSA 1:1, (C) IBP: BSA 3:1 molar ratio.

**Fig. S12** Influence of competitive binding of IBP and CA on BSA thermal denaturation: (A) IBP: BSA 1:1, (B) IBP: BSA 1:1 and CA $3.70 \times 10^{-4}$ M, (C) BSA and CA $3.70 \times 10^{-4}$ M.
**Fig. S13** Influence of competitive binding of IBP and CA on BSA thermal denaturation: (A) IBP: BSA 1:1, (B) IBP: BSA 1:1 and CA $2.10 \times 10^{-3}$ M, (C) BSA and CA $2.10 \times 10^{-3}$ M.

**Fig. S14** Influence of competitive binding of IBP and CA on BSA thermal denaturation: (A) IBP: BSA 0, (B) IBP: BSA 3:1, (C) IBP: BSA 3:1 and CA $3.70 \times 10^{-4}$ M.
**Fig. S15** Temperature variation of the secondary structure content (determined on Dichroweb) for BSA (squares- α-helix, triangles-β-sheets, circles- turns, star-unordered, A-unfolding, B-refolding).
Fig. S16 Temperature variation of the secondary structure content (determined on Dichroweb) for CA: BSA = 1:1 molar ratio (squares- α-helix, triangles-β-sheets, circles- turns, star-unordered, A-unfolding, B-refolding).
Fig. S17 Temperature variation of the secondary structure content (determined on Dichroweb) for CA: BSA = 20:1 molar ratio (squares- α-helix, triangles-β-sheets, circles- turns, star- unordered, A-unfolding, B-refolding).