Thermodynamics stability of bovine plasma fibrinogen in the presence of nanoparticles

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Objectives

- *Estimation of thermodynamic stability of bovine plasma fibrinogen (BPF) in the presence of ZnO NPs and TiO_2 NPs at different phosphate buffer pH values (7.4 and 8) and in aqueous solution.*
  - To evaluate the thermodynamic parameters of the thermal denaturation of bound proteins: denaturation temperature (T_{peak}), heat capacity (ΔC_p) and enthalpy changes (ΔH);
  - To evidence the effect of *pH* values on the BPF denaturation characteristics;
  - To provide insight into adsorption-induced changes in protein structure.

Experimental

Materials

- The nanomaterials used in these investigations are representative nanomaterials from the JRC Repository [1]: TiO_2 (JRCNM01001a) anatase, primary particle size [5-6 nm]; ZnO (JRCNM01100a) uncoated, primary particle size [86 nm].
- Bovine plasma fibrinogen (BPF type I-S), Sigma Aldrich Chemical Company.
- Ultrapure water with conductivity lower than 4.87 µS used for dispersions preparation.

System preparation

Aqueous and phosphate buffer NPs dispersions of BPF (conc.=4 $mgml^{-1}$) in the absence and presence of ZnO NP (JRCNM01100a) (conc. = 0.0115 $mgml^{-1}$) and TiO_2 NP (JRCNM01101a) (conc.=0.00994 $mgml^{-1}$) were sonicated with Bandelin Sonopuls HD 3100 Sonicator for 10 minute; 10% amplitude; energy of 7.192 kJ.

Measurements

NanoDSC (TA Instruments) equipment was used for the evaluation of the thermodynamic parameters of the thermal denaturation of the bound protein in BSA/NPs systems.

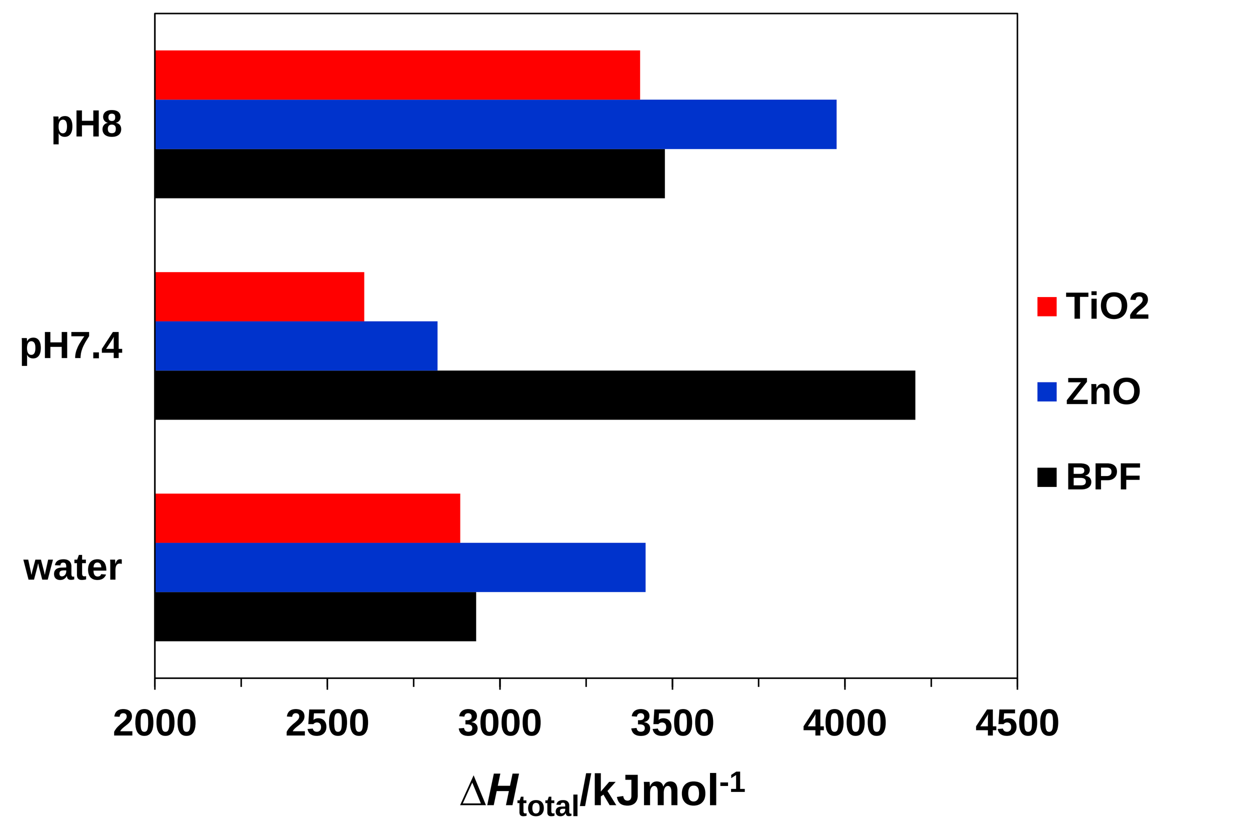
Measurement conditions: pressure 2 atm, temperature range 298 - 378 K, scanning rate of 1 $Kmin^{-1}$. The calorimetric data were corrected using a sigmoidal baseline in NanoAnalyze software.

The calorimetric enthalpy of denaturation (ΔH), denaturation temperature (T_{peak}) and the full width of the transition at half maximum of the transition peak (*FWHM*) were calculated from the DSC data via PeakFit v.4.12 software.

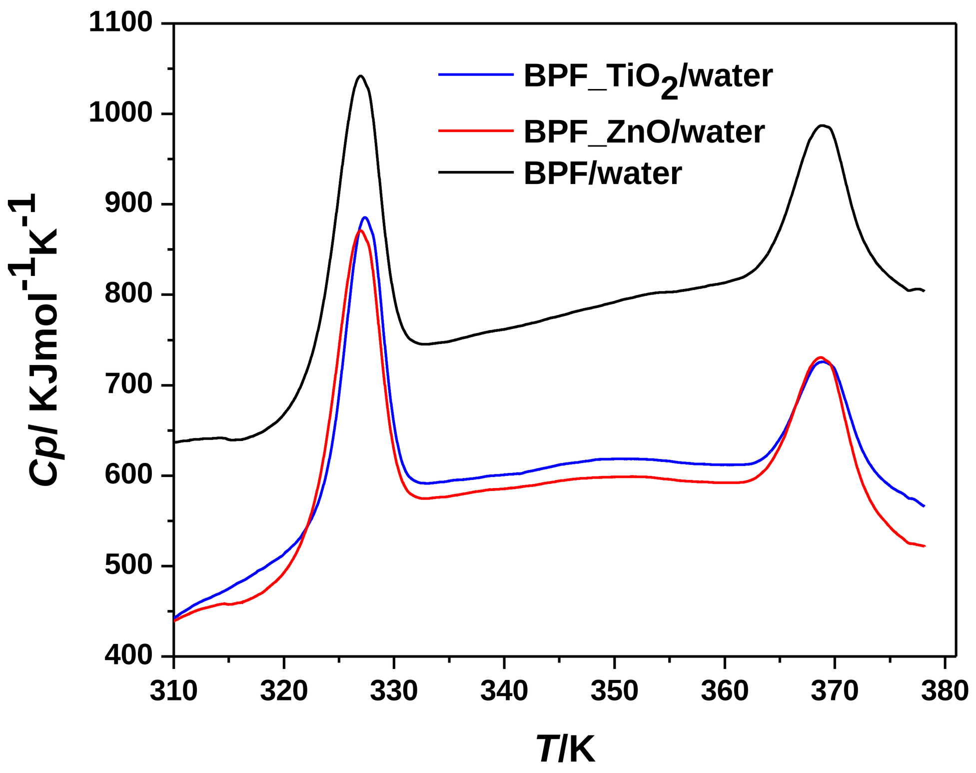
Results

Table 1. Fitting parameters obtained from PeakFit decomposition of DSC signals for thermal denaturation of BPF in different systems

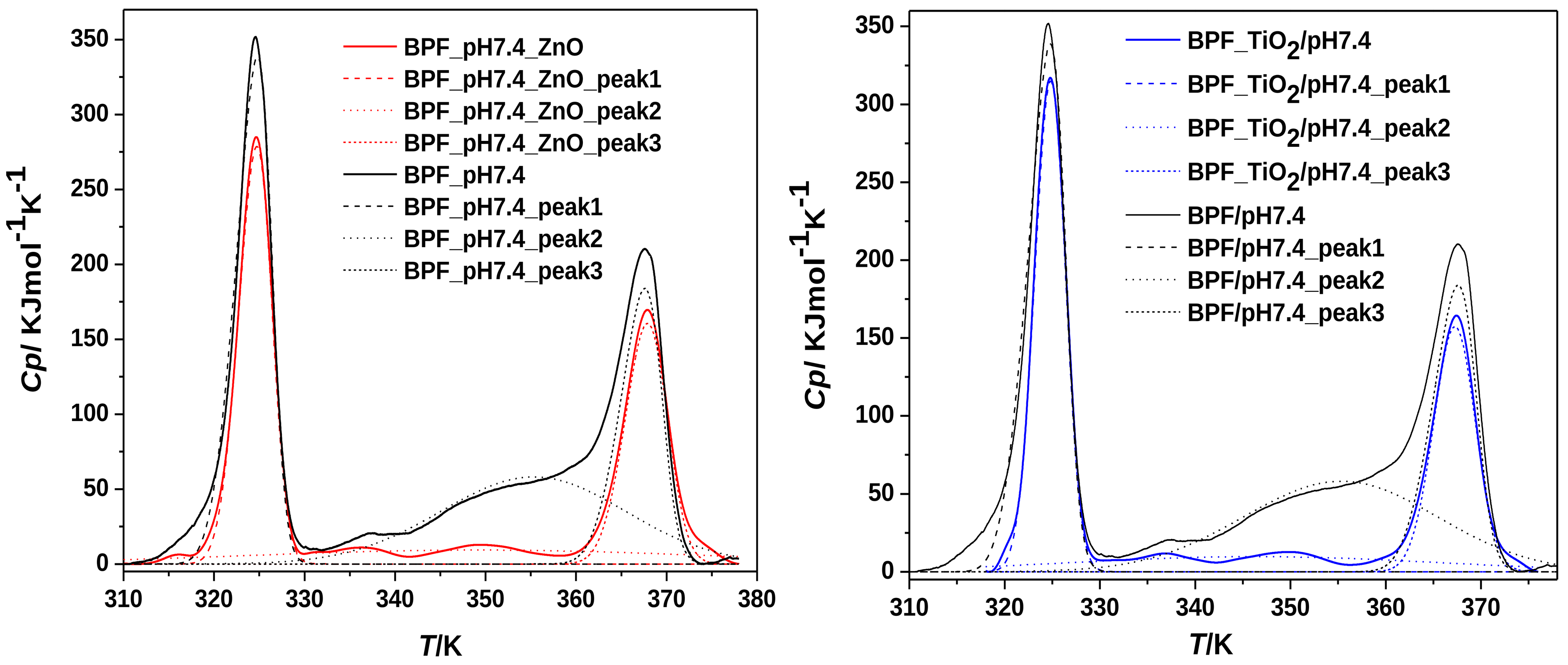
System	BPF/pH 7.4	BPF/pH 8	BPF_ZnO/pH 7.4	BPF_ZnO/pH 8	BPF_TiO_2/pH 7.4	BPF_TiO_2/pH 8
T_{peak1}/K	324.79	324.32	324.72	324.35	324.83	324.41
T_{peak2}/K	355.34	357.13	*349.24*	*346.58*	*346.34*	*343.94*
T_{peak3}/K	367.60	366.23	367.90	366.66	367.27	366.46
$FWHM_{peak1}$/K	4.64	4.35	4.33	4.62	3.80	5.64
$FWHM_{peak2}$/K	23.97	21.47	59.99	66.93	45.27	6.49
$FWHM_{peak3}$/K	5.34	5.79	5.49	6.64	5.16	7.34
ΔH_1/$kJmol^{-1}$	1675.68	1477.23	1286.31	1315.70	1274.09	1910.27
ΔH_2/$kJmol^{-1}$	1482.31	882.74	593.58	1535.61	467.95	116.92
ΔH_3/$kJmol^{-1}$	1046.07	1118.29	939.52	1124.45	864.99	1379.29
r2	0.9894	0.9925	0.9985	0.9895	0.9987	0.9184
F-value	41035.46	53117.14	3.018 e+05	41917.57	2.797e+05	4799.05



Enthalpy change obtained from PeakFit decomposition for thermal denaturation of BPF in the absence and in the presence of ZnO and TiO_2 NPs in phosphate buffer and in water



The raw data of heat capacity versus temperature profiles for the thermal denaturation of aqueos solution of BPF in the presence of NPs



PeakFit decomposition of the thermal denaturation signal of BPF in the presence of NPs at pH 7.4

Conclusions

- Thermal denaturation of BPF presents three components: two narrow peaks attributed to the denaturation of the end D and central E fragments of fibrinogen, and one small and broad peak related to the denaturation of C-terminal [2,3].
- All the thermal transitions of BPF in the absence and in the presence of nanoparticleswere found to be irreversible.
- In water, ZnO NPs produce an increase of total enthalpy change, while the presence of TiO_2 NPs has no a significant effect on the enthalpy.
- At pH 7.4 and 8, ZnO and TiO_2 NPs have a destabilizing effect on BPF structure:
  - At pH 7.4 ZnO and TiO_2 NPs have a significant destabilizing effect for the second component of BPF unfolding, with an important decrease of the T_{peak2} and of the corresponding enthalpy change, ΔH_2, indicating that the stability of the proteins in the adsorbed state is reduced compared to the stability in solution. FWHM for the second component increases considerably suggesting a larger conformational heterogeneity of the surface bound proteins;
  - At pH 8: For the both systems (with ZnO and TiO_2 NPs),the T_{peak2} decreases; In the presence of ZnO NPs the denaturation enthalpy and FWHM increases when the fibrinogen was adsorbed onto NPs, but in the presence of TiO_2 NPs, enthalpy and FWHM decreases significantly for the second component. Apparently, the change of the conformational heterogeneity within the adsorbed protein populations is diminished in the TiO_2 NPs presence at pH 8.
  - In the presence of ZnO and TiO_2 NPs, thetotal enthalpy change for thermal unfolding of protein decreases atpH 7.4; at pH 8, the total enthalpy change increases in the presence of ZnO NPs and slightly decreases in the TiO_2 NPs presence.

References

[1] JRC NANOMATERIALS REPOSITORY, List of Representative Nanomaterials, 2014; 2016

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[3] Chen, Y., Zhang, X., Gong, Y., Zhao, N., Zeng, T., Song, X., Conformational Changes of Fibrinogen Adsorption onto Hydroxyapatite and Titanium Oxide Nanoparticles, Journal of Colloid and Interface Science (1999) 214, 38–45.

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