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THERMODYNAMIC PARAMETERS OF THE PROTEIN THERMAL STABILITY IN THE PRESENCE OF NANOPARTICLES

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Objective

✓ ***Estimation of thermodynamic parameters - denaturation temperature (T_{peak}), heat capacity change (ΔC_p) and enthalpy change (ΔH_{cal}) - describing bovine serum albumin (BSA) and bovine plasma fibrinogen (BPF) stability in the presence of nanoparticles (ZnO, TiO_2) in 0.1 M phosphate buffer pH 7.4***

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 serum albumin (BSA Fraction V) and Bovine plasma fibrinogen (BPF type I-S), phosphate buffer (KH_2PO_4, K_2HPO_4) were purchased from Sigma Aldrich.**
- **Ultrapure water with conductivity lower than 4.87 µS has been used for sample preparation in 0.1M phosphate buffer *pH* 7.4 .**

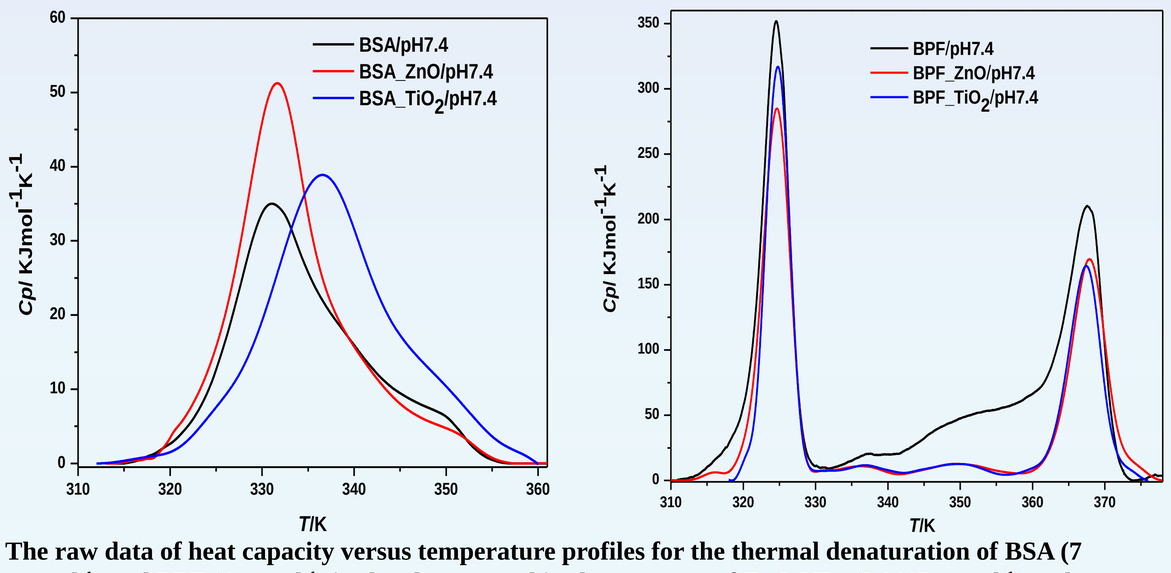
Measurements

***NanoDSC (TA Instruments) equipment* was used for the evaluation of the thermodynamic parameters of the thermal denaturation of free and bound proteins in BSA/NPs and BPF/NPs systems.**

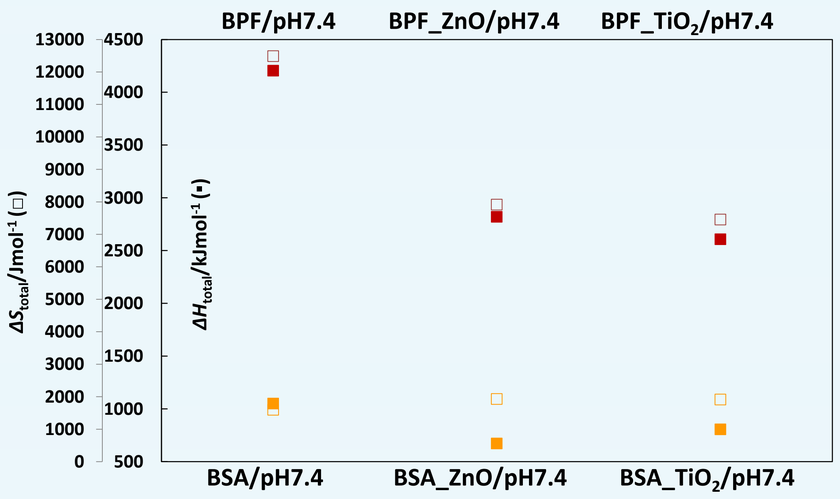
Measurement conditions: pressure 2 atm, temperature range 298 - 378 K, scanning rate of 1 K min^{-1}. The calorimetric data were corrected using a sigmoidal baseline in NanoAnalyze software.

The calorimetric enthalpy change (ΔH_{cal}) and denaturation temperature (T_{peak}) were calculated from decomposed DSC signal obtained *via* PeakFit v.4.12 software.

Results



The raw data of heat capacity versus temperature profiles for the thermal denaturation of BSA (7 $mgml^{-1}$) and BPF (4 $mgml^{-1}$) in the absence and in the presence of ZnO NPs (0.0115 $mgml^{-1}$) and TiO_2 NPs (0.00994 $mgml^{-1}$) in 0.1 M phosphate buffer *pH* 7.4



Total Enthalpy change obtained from PeakFit decomposition and entropy calculation for BSA and BPF unfolding, in the absence and in the presence of ZnO and TiO_2 NPs in 0.1 M phosphate buffer *pH* 7.4

Fitting parameters obtained from PeakFit decomposition of DSC signals of BSA (7 $mgml^{-1}$) and BPF (4 $mgml^{-1}$) in the absence and in the presence of ZnO NPs (0.0115 $mgml^{-1}$) and TiO_2 NPs (0.00994 $mgml^{-1}$) in 0.1 M phosphate buffer *pH* 7.4

System	BSA/pH7.4	BSA_ZnO/pH7.4	BSA_TiO_2/pH7.4	BPF/pH7.4	BPF_ZnO/pH7.4	BPF_TiO_2/pH7.4
T_{peak1} ± S.D. /K	331.15±0.57	331.48 ±0.05	336.76 ±0.22	324.79±0.03	324.72±0.01	324.83±0.01
T_{peak2} ± S.D. /K	343.52±1.11	335.45 ±0.03	345.74.±0.25	355.34±0.1	349.24±0.34	346.34±0.27
T_{peak3}± S.D. /K	-	-	-	367.60±0.03	367.90±0.1	367.27±0.01
$\Delta H_{cal\ 1}$ ± S.D. /kJmol^{-1}	397.29±15.76	280.09±1.47	484.73±7.1	1675.68±3.35	1286.31±1.27	1274.09±1.47
$\Delta H_{cal\ 2}$ ± S.D. /kJmol^{-1}	133.99±15.9	366.89±1.64	158.05±7.07	1482.31±12	593.58±8	467.95±7.49
$\Delta H_{cal\ 3}$ ± S.D. /kJmol^{-1}	-	-	-	1046.07±7.2	939.52±1.73	864.99±1.93
$\Delta H_{cal\ total}$/kJmol^{-1}	531.29	646.98	642.79	4204.06	2819.41	2607.04
r2	0.9954	0.9976	0.9983	0.9894	0.9985	0.9987
ΔS_{total} ± S.D. /Jmol^{-1}	1595.86±0.01	1927.97±0.01	1911.59±0.01	12483±0.06	7912.9±0.05	7452.61±0.06
$C_{P\ max\ peak1}$/kJmol^{-1} K^{-1}	33.23	34.57	38.29	338.7	278.61	314.88
$C_{P\ max\ peak2}$ /kJmol^{-1} K^{-1}	9.85	17.67	13.92	58.09	9.29	9.71
$C_{P\ max\ peak3}$ /kJmol^{-1} K^{-1}	-	-	-	183.87	160.51	157.25
$\Delta H_{vH\ peak\ 1}$/ kJmol^{-1}	305.05	451.04	297.94	709.34	759.57	867.27
$\Delta H_{vH\ peak\ 2}$/kJmol^{-1}	288.51	180.24	350.14	164.57	63.49	82.78
$\Delta H_{vH\ peak\ 3}$/kJmol^{-1}	-	-	-	789.95	769.05	815.54
C.U. $_{peak\ 1}$	0.77	1.61	0.61	0.42	0.59	0.68
C.U. $_{peak\ 2}$	2.15	0.49	2.21	0.11	0.11	0.18
C.U. $_{peak\ 3}$	-	-	-	0.76	0.82	0.94

ΔH_{cal} – the calorimetric enthalpy
ΔH_{vH} – the van't Hoff enthalpy
S.D. – The best fit standard error
C.U.– cooperative unit

$\Delta H_{vH} = 4RT^2_{peak}\ C_{P\ max}/\Delta H_{cal}$
C.U.= $\Delta H_{vH}/\Delta H_{cal}$
R=8.3145 Jmol^{-1} K^{-1}

Conclusions

✓**The thermodynamic parameters of the thermal fingerprint of the protein have been evaluated. The calorimetric data indicate severe structural rearrangements of BSA and BPF bound to NPs.**

✓**The denaturation enthalpies for BPF adsorbed on NPs were strongly reduced with respect to those of protein in solution.**

➢**ZnO and TiO_2 NPs have different effects on BSA thermal stability at *pH* 7.4 :**

- **ZnO NPs produce a major destabilization of the second component of BSA thermal unfolding;**
- **TiO_2 NPs have a stabilizing effect on both components of BSA denaturation, thermal denaturation of protein appearing at higher temperatures and the corresponding enthalpy change have larger values than BSA free in solution.**
- **The heat capacity of the second component of BSA thermal denaturation in the presence of ZnO decreases possibly due to electrostatic repulsion between the negatively charged BSA and the negatively charged ZnO NPs.**

➢**At *pH* 7.4, ZnO and TiO_2 NPs have a destabilizing effect on BPF structure:**

- **ZnO and TiO_2 NPs have a significant destabilizing effect, more pronounced for the second component of BPF unfolding in the presence of TiO_2 NPs, 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.**
- **In the presence of ZnO and TiO_2 NPs at *pH* 7.4, the total enthalpy change decreases.**
- **The nanoparticles surface can induce conformational changes in adsorbed protein molecules and can also introduce thermodynamic instability making it susceptible to chemical denaturation which may affect the overall bio-reactivity of the nanoparticle [2].**
- **Changes in the C_p are believed to originate from the disruption of the forces stabilizing native protein structure [3].**
- **Thermal denaturation of BPF presents three components: two narrow peaks attributed to the denaturation of the end D and central E fragments of fibrinogen, respectively, and one small and broad peak related to the denaturation of C-terminal [4,5].**

➢**For a first-order two-state transition, the van't Hoff enthalpy is equal to the calorimetric enthalpy, ΔH_{cal}. In other words, the heat effect for the transition A → B is the calorimetric enthalpy, which correspondingly governs the distribution between the two phases [6].**

- **If $\Delta H_{vH} < \Delta H_{cal}$ the process involves one or several intermediate stages, such as A → B → C and is called non-two state.**
- **If $\Delta H_{vH} > \Delta H_{cal}$ the process involves cooperativity (C.U.), but is not "completely cooperative" as in a first order transition.**
- **The distribution of molecules between the two phases is much more temperature dependent than the actual heat effect of the phase transition due to cooperative motion of the molecules. Therefore, for a non-two-state transition or a partially cooperative transition there are two separate enthalpy parameters, ΔH_{vH} and ΔH_{cal}.**

✓**DSC provides information on the temperature dependence of the heat capacity over a broad temperature range leading to thermograms.**

References

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Acknowledgements

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