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The study of the protein – nanoparticles interaction by calorimetric methods

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Objectives

- ***Evaluation of native protein - nanoparticles (TiO_2 and SiO_2) interaction;***
- ***Study of TiO_2 and SiO_2 nanoparticles (NPs) influence on the thermal stability of bovine serum albumin (BSA) in water;***
- ***Estimation of thermodynamic parameters: denaturation temperature (T_{peak}), heat capacity change (ΔC_p) and enthalpy change (ΔH) describing the protein stability in the presence of NPs.***

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]; SiO_2 (JRCNM10404a), primary particle size [58 nm].
•Bovine serum albumin (BSA) was purchased from Sigma Aldrich Chemical Company.
•Ultrapure water with conductivity lower than 4.87 µS has been used for NPs dispersions and protein solutions preparation.

System preparation

Aqueous NPs dispersions with different concentrations: TiO_2 NPs (0.00994 mg ml^{-1}) and SiO_2 (0.0058 mg ml^{-1}) were sonicated with Bandelin Sonopuls HD 3100 Sonicator for 10 minute; 10% amplitude; energy of 7.192 kJ.

Measurements

ITC200 microcalorimeter (MicroCal Inc.) **was used for the analysis of binding characteristics for protein-NPs systems: stoichiometry *n*, the binding constant *K*, enthalpy ΔH and entropy ΔS changes. The titration experiments were performed at constant temperature, 298 K.**
NanoDSC (TA Instruments) equipment **was used to study thermal denaturation behavior of protein in solution, in the absence and presence of NPs. 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 far-UV circular dichroism (CD) spectra were measured in order to evaluate the changes of the protein secondary structure induced by the presence of NPs. The CD spectra were recorded on a JASCO J-815 CD spectropolarimeter, at 298 K.

Results

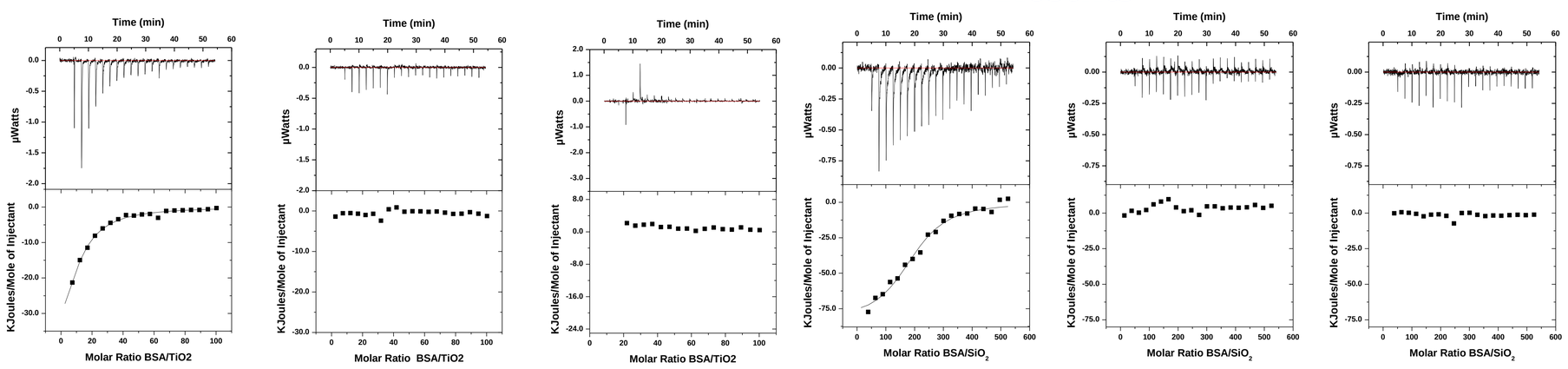

Fig. 1 (A) ITC thermograms for the interaction of BSA with TiO_2 NPs in water. The continuous line in the lower panel represents the fitting of the data, after the subtraction of dilution effects of TiO_2 NPs (B) and BSA (C). [BSA] = 5.12x10^{-4} M; [TiO_2 NPs] = 9x10^{-7} M.

Fig. 2 (A) ITC thermograms for the interaction of BSA with SiO_2 NPs in water. The continuous line in the lower panel is obtained by fitting of the data, after the subtraction of dilution effects of SiO_2 NPs (B) and BSA (C). [BSA] = 1.0x10^{-4} M; [SiO_2 NPs] = 3.35x10^{-8} M.

Table 1. Thermodynamic parameters of BSA interaction with TiO_2 and SiO_2 NPs in pure water.

System	n	K M^{-1}	ΔH kJ mol^{-1}	ΔS J mol^{-1} K^{-1}	ΔG kJ mol^{-1}
BSA-TiO_2	9.14± 1.07	1.09x10^5 ± 9.43x10^3	-61.09± 8.16	-108± 27.3	-28.7± 0.2
BSA-SiO_2	195± 7.32	1.28x10^6 ± 2.63x10^5	-83.76± 4.42	-164± 14.9	-34.8± 0.5

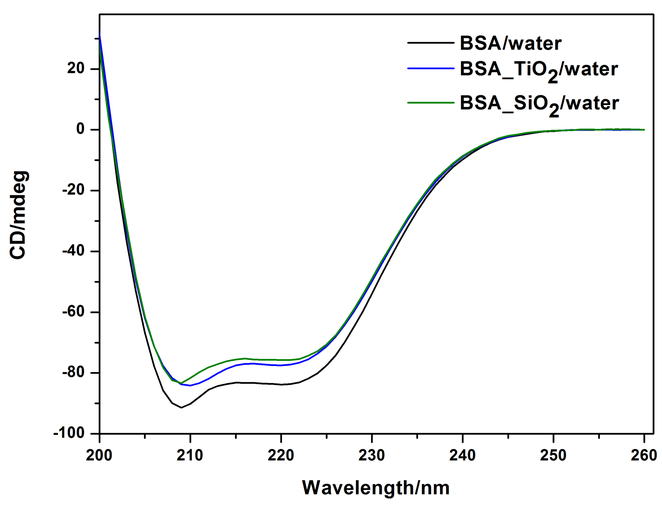

Fig. 3 CD spectra for BSA (0.049 mg ml^{-1}) in absence and in the presence of TiO_2 NPs (0.000071 mg ml^{-1}) and SiO_2 NPs (0.0000406 mg ml^{-1}) in water

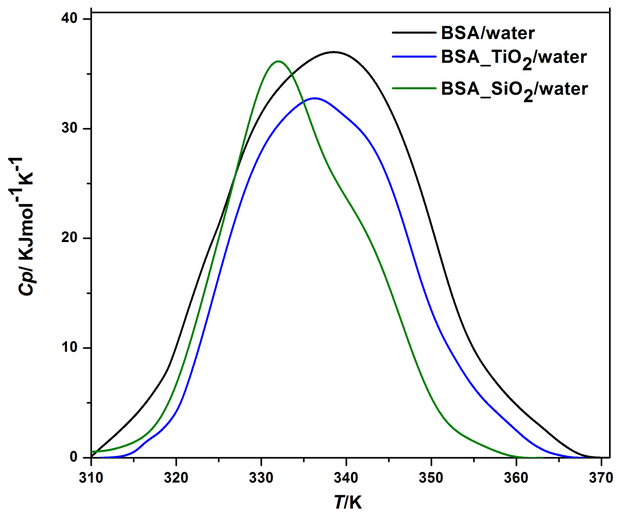

Fig. 4 DSC scans of the thermal denaturation of BSA (7mg ml^{-1}) in the presence of TiO_2 NPs (0.00994 mg ml^{-1}) SiO_2 NPs (0.0058 mg ml^{-1}) in water

PeakFit decomposition of DSC signals of BSA (7mg ml^{-1}) in the absence and the presence of TiO_2 NPs (0.00994 mg ml^{-1}) and SiO_2 NPs (0.0058 mg ml^{-1}) in water and the value of calculated ΔS_{total} .

System	BSA/water	BSA_TiO_2/water	BSA_SiO_2/water
T_{peak1}/K	332.93	331.33	330.57
T_{peak2}/K	345.03	342.82	340.37
FWHM $_{peak1}$/K	22.03	16.56	12.33
FWHM $_{peak2}$/K	17.06	16.69	13.65
ΔH_1 /kJmol^{-1}	754.73	469.15	400.38
ΔH_2/kJmol^{-1}	294.97	336.62	303.75
r2	0.9991	0.9991	0.9997
F-value	6.8521e+05	6.2976e+05	1.5144e+06
ΔS_1/Jmol^{-1} K^{-1}	2252.43	1409.74	1216.41
ΔS_2/Jmol^{-1} K^{-1}	850.44	977.14	891.81
ΔS_{total} /Jmol^{-1} K^{-1}	3102.87	2386.88	2108.22
$C_{P\,max\ peak1}$/ kJmol^{-1} K^{-1}	32.18	26.61	35.06
$C_{P\,max\ peak2}$/ kJmol^{-1} K^{-1}	16.23	18.94	23.21
$\Delta H_{vH\ peak\ 1}$/ kJ mol^{-1}	157.20	207.07	318.07
$\Delta H_{vH\ peak\ 2}$/ kJ mol^{-1}	217.83	219.91	294.25
C.U. peak 1	0.21	0.44	0.79
C.U. peak 2	0.74	0.65	0.97

Conclusions

✓ITC measurements: ΔG<0 shows the spontaneity of BSA binding to the surface of NPs. The large and favorable value of the enthalpy and the unfavorable contribution of the entropy, (ΔH<0, ΔS<0), suggest that the binding of the BSA on TiO_2 and SiO_2 NPs surface is an entirely enthalpy-controlled process.
✓DSC measurements: all the thermal transitions of BSA in water, free and in the presence of NPs were found to be irreversible. The irreversibility is probably due to aggregation of the unfolded protein chains. It is agreed that such an aggregation involves a relatively small enthalpy change, which means that the effect recorded by DSC represents essentially the enthalpy change of the unfolding process. The thermodynamic parameters of the thermal fingerprint of the protein have been evaluated. Calorimetric data indicate severe structural rearrangements of BSA bound to NPs. The shape of the unfolding thermograms also revealed the non-uniform character of the protein population due to surrounding water.
✓CD spectra: a decrease in the α-helix content in the presence of NPs and the increase of β-sheet and random coil fractions.

Reference

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

Acknowledgements

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