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Thermodynamic study of protein stability in the presence of SiO_2 nanoparticles

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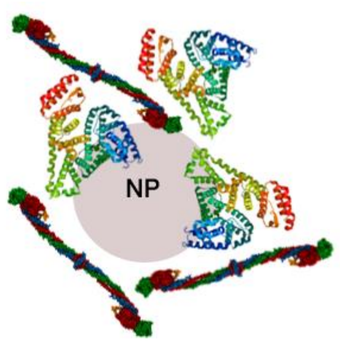
Objectives

- The study of the complex phenomena occurring at the interaction of nanoparticles (NPs) with proteins by evaluating the effect of the NPs on the protein stability and the thermodynamics of binding interactions.
- Correlation between thermodynamic properties and the dominant contributions determining adsorption processes during NPs/proteins interactions using the equipment NanoDSC TA Instruments.
- Evaluation of native protein-nanoparticle interaction by using ITC200 microcalorimeter (MicroCal Inc.).



Sample preparation and caracterization

The nanomaterial used in this work was SiO_2 NPs (Reference Nanomaterial JRCNM10404a) [1]. The study has been performed with bovine serum albumin (BSA) and bovine plasma fibrinogen (BPF type I-S) at 3 different concentrations (0.0058, 0.058 and 0.14 $mgml^{-1}$) of SiO_2 NPs in water. The concentration of proteins in water solution was: c_{BSA}=7 $mgml^{-1}$ and c_{BPF}=4 $mgml^{-1}$. NPs dispersions were sonicated with Bandelin Sonopuls HD 3100 Sonicator system for 10 minute with 10% amplitude and energy of 7.192 kJ.

NanoDSC TA Instruments equipment was used for measurements of thermal denaturation behaviour of proteins in water, in the absence and in the presence of nanoparticles, in the temperature range 279 K to 378 K, with a scanning rate of 1 $Kmin^{-1}$. The measurements were made at constant pressure of 2 atm.

The quantitative thermodynamic parameters: heat capacity change (ΔC_p), denaturation temperature (T_{peak}) and denaturation enthalpy (ΔH) of the bound proteins in the BSA/SiO_2 and BPF/SiO_2 systems were evaluated.

Analysis of binding characteristics for protein-NPs systems has been done by using Isothermal Titration Calorimetry with Microcal iTC200 equipment. The thermodynamic data have been evaluated to get insight into adsorption-induced changes in the protein structure and stability, as well as into mechanism of binding interaction.

Results and discussion

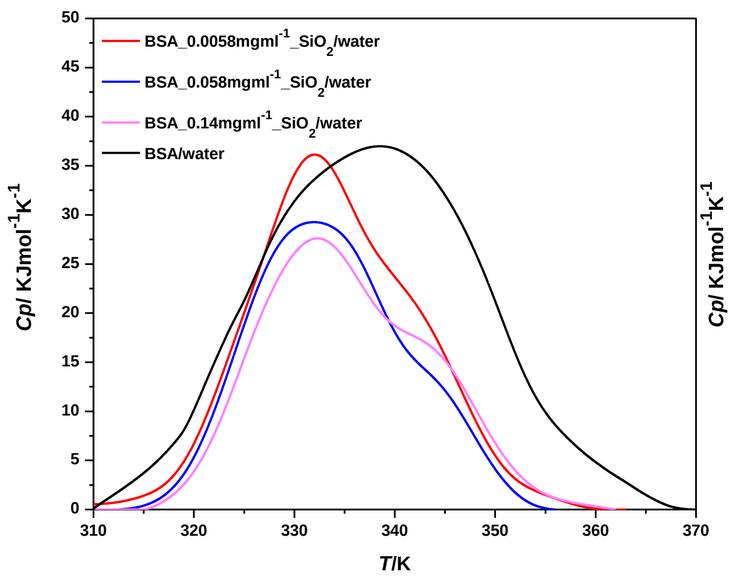


Fig. 1 The heat capacity versus temperature profiles for the thermal denaturation of BSA in the absence and presence of SiO_2 NPs at different concentrations in water

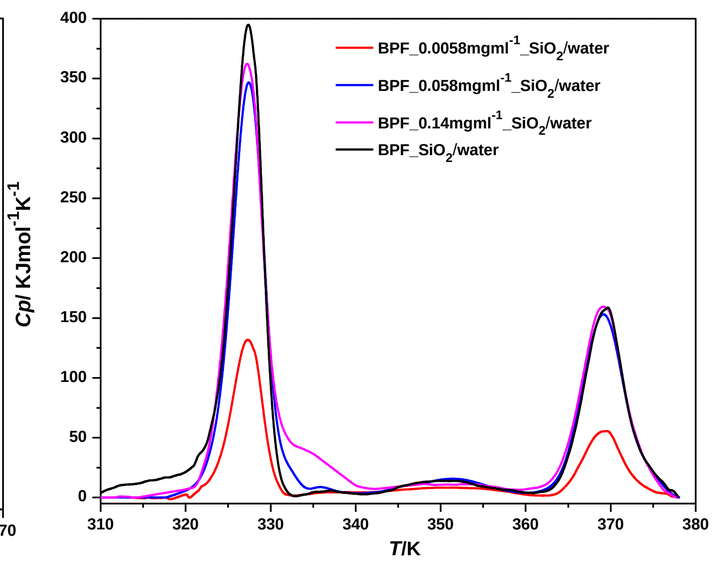


Fig. 2 The heat capacity versus temperature profiles for the thermal denaturation of BPF in the absence and presence of SiO_2 NPs at different concentrations in water

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

System	BSA /water	BSA_SiO_2 /water	BSA_SiO_2 /water	BSA_SiO_2 /water
c/$mgml^{-1}$	7	0.0058	0.058	0.14
T_{peak1}/K	332.93	330.57	330.96	331.02
T_{peak2} /K	345.03	340.37	345.16	343.25
$FWHM_{peak1}$/K	22.03	12.33	16.33	13.09
$FWHM_{peak2}$/K	17.06	13.65	15.17	13.26
ΔH_1 /$kJmol^{-1}$	754.73	400.38	481.54	359.9
ΔH_2 /$kJmol^{-1}$	294.97	303.75	98.52	209.2
ΔH_{total}/$kJmol^{-1}$	1049.71	704.13	580.07	569.10
r2	0.9991	0.9997	0.9987	0.9993
F-value	6.8521e+05	1.5144e+06	2.8863e+05	6.9795e+05

Table 2. Thermodynamic parameters of the interaction of BPF with SiO_2 NPs in pure water

System	BPF/ water	BPF_SiO_2 /water	BPF_SiO_2 /water	BPF_SiO_2 /water
c/$mgml^{-1}$	7	0.0058	0.058	0.14
T_{peak1}/K	327.52	327.45	327.41	327.28
T_{peak2}/K	350.29	348.85	349.76	349.75
T_{peak3}/K	369.54	369.36	369.28	369.2
$FWHM_{peak1}$/K	4.26	4.23	4.41	5.34
$FWHM_{peak2}$/K	13.31	19.32	54.73	16.07
$FWHM_{peak3}$/K	5.87	5.52	5.69	6.07
ΔH_1/$kJmol^{-1}$	1775.07	591.86	1580.51	1954.41
ΔH_2/$kJmol^{-1}$	196.34	165.96	550.22	160.24
ΔH_3/$kJmol^{-1}$	959.75	321.86	867.77	1013.12
ΔH_{total}/$kJmol^{-1}$	2931.16	1079.71	3007.5	3127.78
r2	0.9896	0.9965	0.9968	0.9754
F-value	42423.59	1.2009e+05	1.3816e+05	8766.68444

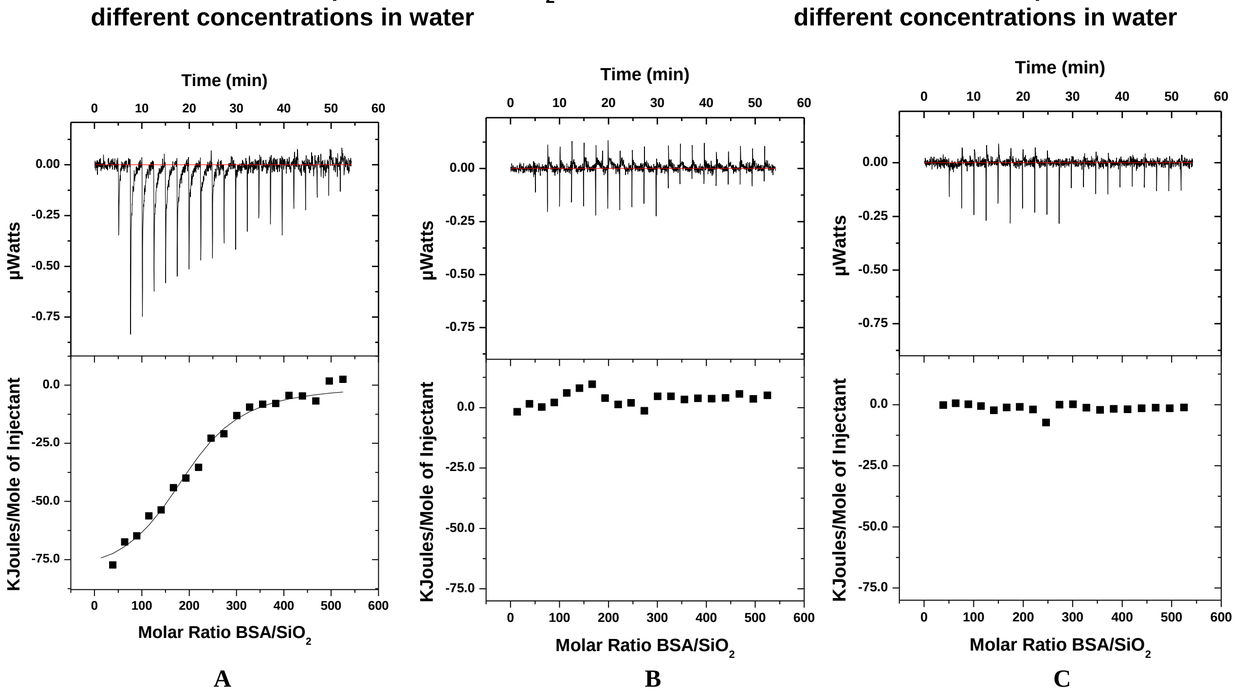


Fig. 3 (A) ITC thermograms for the interaction of BSA with SiO_2 NPs in water. The continuous line in the lower panel is obtained by fitting the data after the subtraction of dilution effects of SiO_2 NPs (B) and BSA (C). [BSA] = $1.0x10^{-4}$ M; [SiO_2 NPs] = $3.35x10^{-8}$ M.

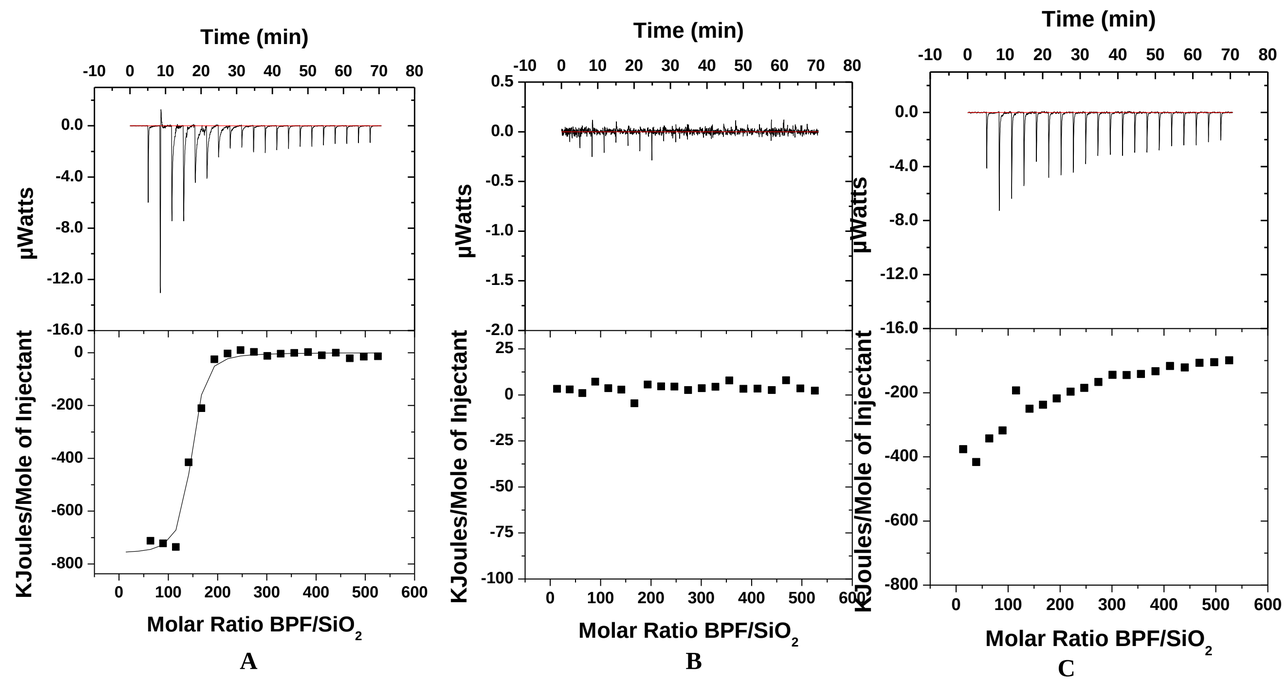


Fig. 4 (A) ITC thermograms for the interaction of BPF with SiO_2 NPs in water. The continuous line in the lower panel is obtained by fitting the data after the subtraction of dilution effects of SiO_2 NPs (B) and BPF (C). [BPF] = $1.0x10^{-4}$ M; [SiO_2 NPs] = $3.35x10^{-8}$ M.

Table 3. Thermodynamic parameters of the interaction of BSA and BPF with 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} K^{-1}
BSA-SiO_2	195±7.32	$1.28x10^6$±$2.63x10^5$	-83.76±4.42	-164±14.9	-34.8±0.5
BPF-SiO_2	136±2.21	$2.55x10^7$±$8.26x10^6$	-762.2±23.63	-2410±79.3	-42.27±0.8

References

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

Conclusions

- All the thermal transitions of BSA and BPF 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.
- The binding of BPF to SiO_2 NPs is stronger than the binding of BSA, the binding constant, K, being higher by an order of magnitude.
- $\Delta G<0$ show the spontaneity of BSA and BPF binding to surface of SiO_2 NPs. The large and favorable value of the enthalpy and the unfavorable contribution of the entropy, ($\Delta H<0$, $\Delta S<0$), show that the binding of the BSA and BPF on SiO_2 NPs surface is an entirely enthalpy-controlled process.

Acknowledgements

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