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THERMODYNAMIC CHARACTERIZATION OF TWO PLASMA PROTEINS ADSORPTION TO SOME NANOPARTICLES

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Objectives

❖The study of the adsorption thermodynamic mechanism of the native protein on the surface of nanoparticles by Isothermal Titration Calorimetry.

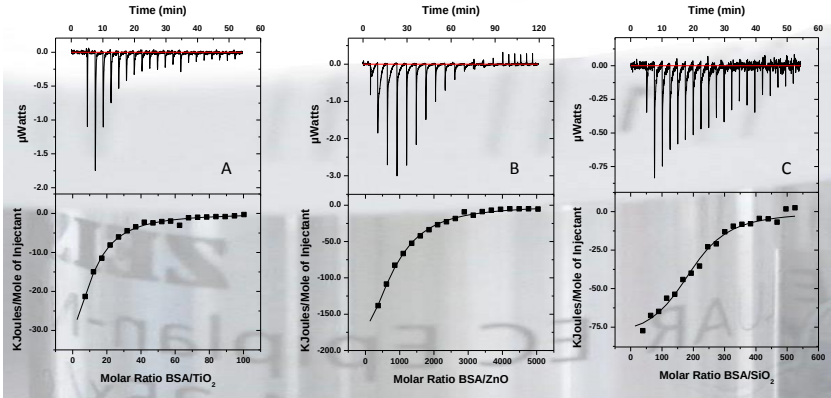
❖The study of the thermal unfolding process of the protein free and adsorbed onto different types of NPs by Differential Scanning Calorimetry.

❖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.

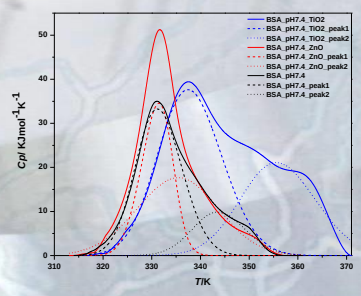
Sample preparation and characterization

Water- or buffer-dispersion of TiO_2, ZnO and SiO_2 NPs at different concentrations and solutions of BSA and BPF were prepared by weight and by adding deionized water or phosphate buffer ($KHPO_4$; Na_2HPO_4) 0.1M at different pH values. The NPs dispersions were sonicated using Bandelin Sonopuls HD 3100 Sonicator systems for 10 minutes with 10% amplitude and an energy of 7.192 kJ. Analysis of binding characteristics for protein-NPs systems has been done at constant temperature of 298 K by using the Microcal iTC200 equipment. The thermal denaturation of protein in the presence of NPs was studied using NanoDSC calorimeter. All DSC measurements were done at constant pressure of 2 atm in the temperature range of 279 K to 378 K, with a scanning rate of 1 $Kmin^{-1}$.

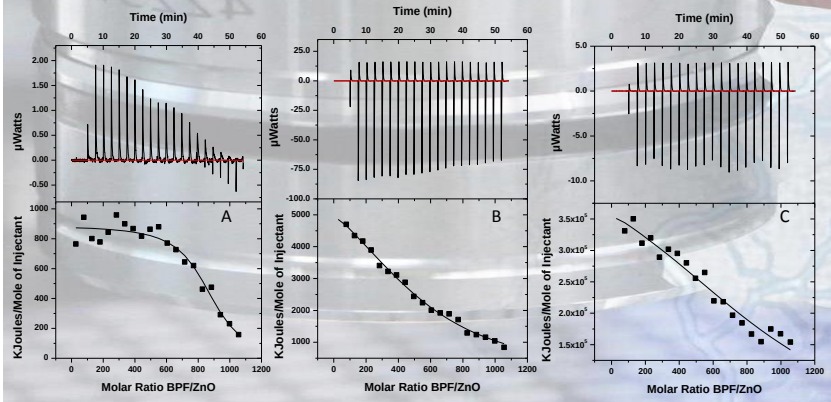
Results and discussion



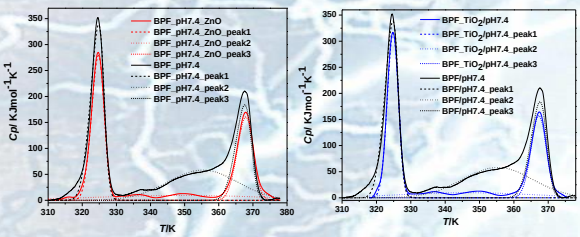
ITC thermograms for the interaction of BSA with (A) TiO_2 NPs (B) ZnO NPs (C) SiO_2 NPs in water. The continuous line in the lower panel is obtained by fitting the data, after the subtraction of dilution effects of the NPs and BSA. The concentrations: (A) TiO_2 NPs = $9x10^{-7}$ M and BSA = $5.12x10^{-4}$ M (B) ZnO NPs = $1.79x10^{-11}$ M and BSA = $5.12x10^{-4}$ M (C) SiO_2 NPs = $3.35x10^{-8}$ M and BSA = $1x10^{-4}$ M.



Comparative Peak Fit decomposition of the thermal signals: BSA (concentration = 7mg/ml) in the absence (black solid line) and in the presence of TiO_2 NP concentration = 0.00994 mg/ml (red line) or ZnO NP concentration = 0.0115 mg/ml (blue line) at three pH 7.4 buffer phosphate. Dash lines represent the corresponding PeakFit components.



ITC measurements for the interaction of BPF with ZnO NPs (A) in water (B) in phosphate buffer pH=7.4 (C) in phosphate buffer pH=8.0. The continuous line in the lower panel is obtained by fitting of the data, after the subtraction of dilution effects of ZnO NPs and BPF. The concentrations were: (A) BPF = $1.75x10^{-5}$ M and ZnO NPs = $2.92x10^{-9}$ M (B) BPF = $1.75x10^{-5}$ M and ZnO NPs = $2.92x10^{-9}$ M (C) BPF = $1.75x10^{-5}$ M and ZnO NPs = $2.92x10^{-9}$ M.



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

Conclusions

➢The ITC measurements show that the adsorption of different proteins on the surface of the same nanoparticle can occur through a different mechanism.
ΔG<0 shows the spontaneity of BSA binding to 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, ZnO and SiO_2 NPs surface is an entirely enthalpy-controlled process. The thermal effects of the ZnO and TiO_2 NPs interaction with BSA at pH=7.4 and pH=8.0 were too small to be analyzed. Also, the thermal effects of the BPF interaction with TiO_2 NPs in water and at pH=7.4 and pH=8.0 were too small.

➢For the interaction of BPF with ZnO NPs, ΔG<0 corresponds to the spontaneous binding of BPF to ZnO NPs in all studied environments. The unfavorable enthalpy and the large and favorable value of entropy, (ΔH>0, ΔS>0), indicate that the binding of the BPF on ZnO NPs surface is an entirely entropy-controlled process.

➢The DSC measurements show that at pH 7.4 TiO_2 NPs have a stabilizing effect on BSA structure, thermal denaturation of protein appearing at higher temperatures. The major effect produced by ZnO NPs presence at pH 7.4 is a destabilizing one on the second component of BSA thermal denaturation.

➢At pH 7.4 ZnO and TiO_2 NPs have a significant destabilizing effect for the second component of BPF unfolding, the stability of the protein 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.

Acknowledgements

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