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The effect of crystalline structure and surface characteristics of different TiO_2 nanoforms on bovine serum albumin thermal denaturation: a differential scanning calorimetry study

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HIGHLIGHTS

❖ The change of BSA thermal stability taking place during adsorption process on TiO_2 nanoparticles of different sizes was investigated by means differential scanning calorimetry. The thermodynamic parameters of the thermal fingerprint of the bound protein represented by heat capacity change, denaturation temperature and enthalpy, as well as heat capacity change of the bound protein were evaluated.

❖ Two thermodynamically independent but interacting units of BSA were examined: component 1 consisting of domain III and most of domain II, which melts at lower temperatures and component 2 composed of domain I and a small part of domain II, unfolding at higher temperature.

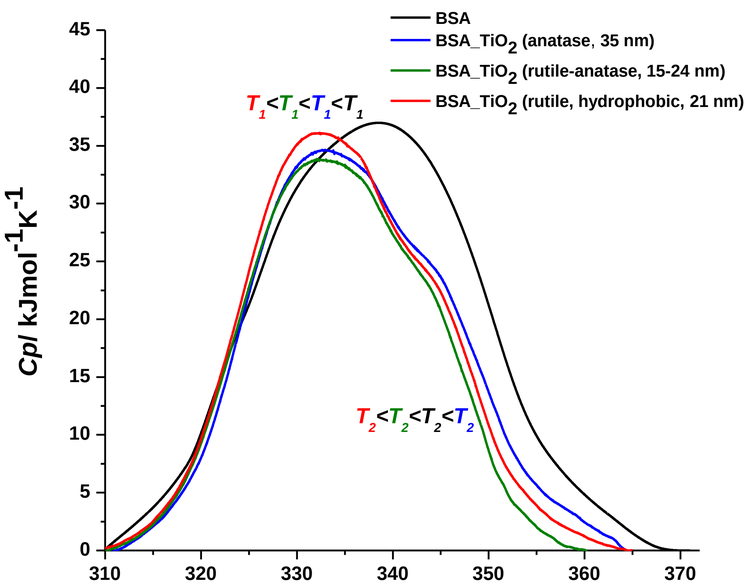
Materials

• Three types of titania nanoparticles (TiO_2 NPs) (anatase; rutile hydrophobic, Al-coated; rutile-anatase) with various particle sizes (35; 21; 15-24 nm). They are representative nanomaterials from the JRC Repository [1]; • Bovine Serum Albumin (BSA) was purchased from Sigma Aldrich Chemical Company; • Ultrapure water (18.2 MΩ cm Millipore) and phosphate buffer (KH_2PO_4 and Na_2HPO_4) pH 7.4 for NPs dispersions and protein solutions preparation.

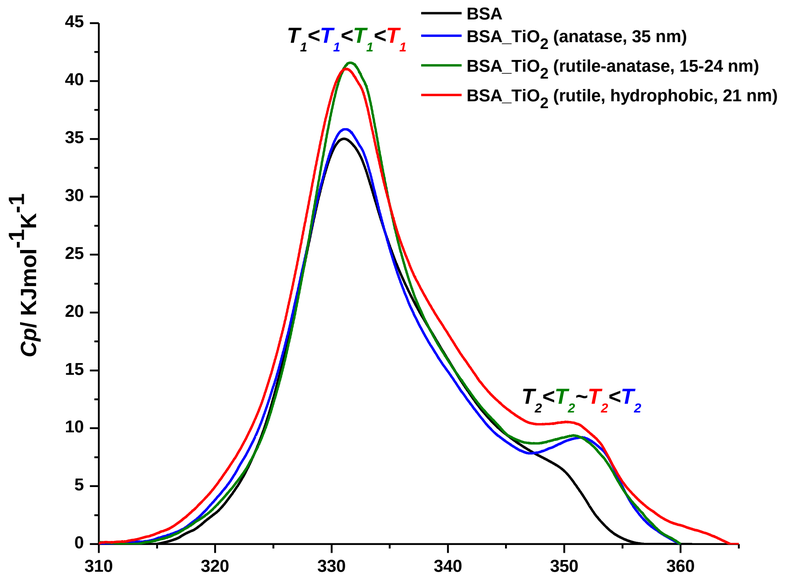
Experimental Technique

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 298368 K, scanning rate of 1 K min^{-1}. The raw data were processed using a sigmoidal baseline in NanoAnalyze software. Calorimetric denaturation enthalpy (ΔH_{cal}) and temperature (T_{peak}) were otained from the DSC data decomposition via PeakFit v.4.12 software; van't Hoff enthalpy change (ΔH_{vH}) was calculated by: $\Delta HvH = 4\,R\,T_{peak}^2\,\frac{Cp_{max}}{\Delta Hcal}$

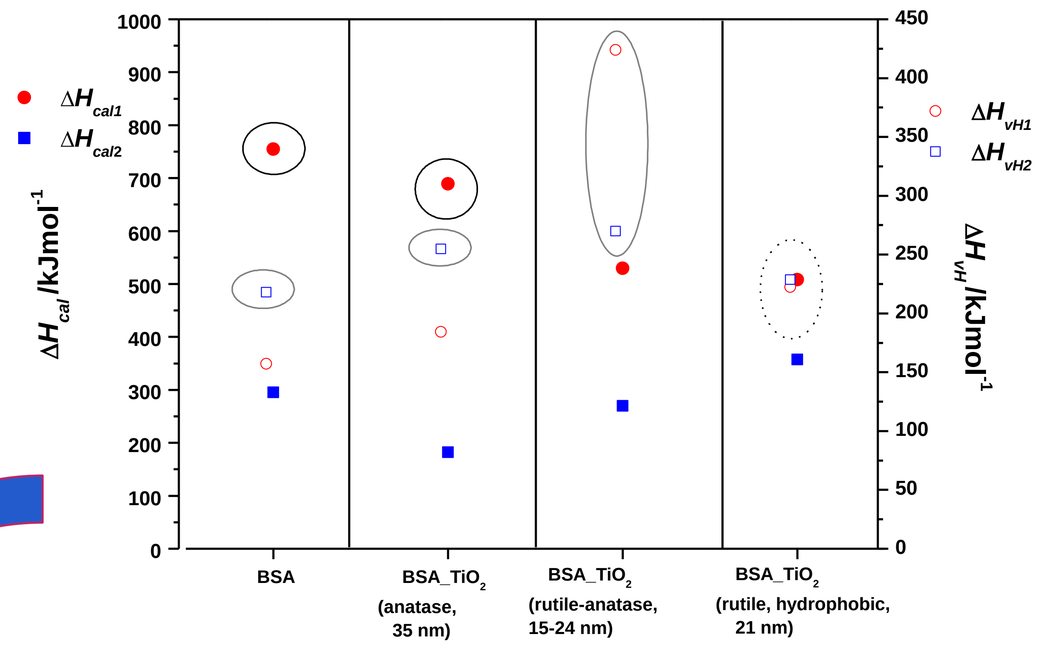
RESULTS



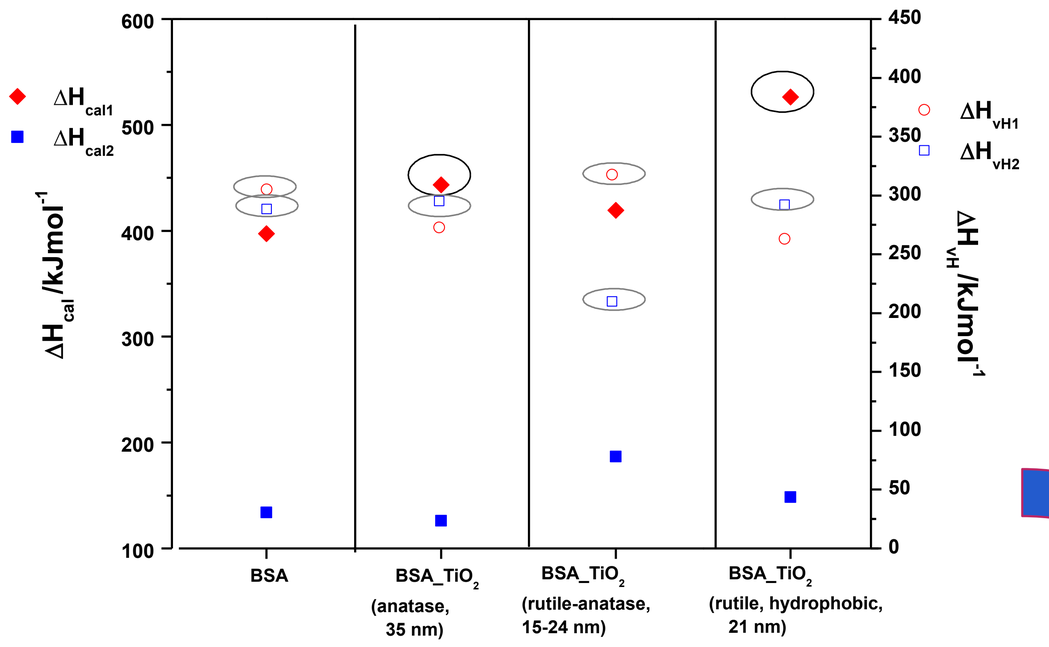
DSC scans of BSA (7 mg ml^{-1}) thermal denaturation in the absence and in the presence of TiO_2 NPs (0.01 mg ml^{-1}), in water



DSC scans of BSA (7mg ml^{-1}) thermal denaturation in the absence and in the presence of TiO_2 NPs (0.01 mg ml^{-1}) at pH7.4



Enthalpy of thermal denaturation of BSA in the absence and in the presence of TiO_2 NPs (0.01 mg ml^{-1}), in water



Enthalpy of thermal denaturation of BSA in the absence and in the presence of TiO_2 NPs (0.01 mg ml^{-1}) at pH7.4

$\Delta H_{cal} > \Delta H_{vH}$: the unfolding is not a simple 2-state process, it involves intermediates or independent domains for component 1 of BSA and BSA-TiO_2 anatase (water); BSA-TiO_2 anatase, BSA-TiO_2 rutile (pH 7.4).

$\Delta H_{vH} > \Delta H_{cal}$: the data suggest the presence of a possible cooperative intermolecular process involved in the thermal unfolding: component 2 for all systems in water and pH 7.4.

CONCLUSIONS

✓ *A strong dependence of protein stability on crystalline structure and particle surface characteristics of the titanium oxide, as well as on the physiological environment was observed:*

- In water the different TiO_2 nanoforms (rutile, 21 nm and rutile-anatase, 15-24 nm) have a destabilizing effect on the thermal unfolding of BSA: lower values for the transition temperature of peaks were observed for NPs-adsorbed protein. ΔH_{total} for BSA denaturation decreases strongly in the presence of NPs, indicating that the stability of the protein in the adsorbed state is reduced compared to the stability of free BSA;
- In phosphate buffer pH 7.4, all different TiO_2 nanoforms have a stabilizing effect on the thermal unfolding of BSA: higher values for the transition temperature of both peaks were observed for NPs-adsorbed protein.

✓ *The results of the calorimetric investigation contribute to a better understanding of the changes in structural stability of the protein following adsorption process of nanomaterials.*

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

Acknowledgements
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