

SUPPORTING INFO

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Conformational analysis of bovine serum albumin adsorbed on Halloysite Nanotubes and Kaolinite: a Fourier Transform Infrared Spectroscopy study.

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Peak fitting procedure for the quantitation of the BSA secondary structure.

Tables S1-S8 show the quantitative results of the secondary structure analysis of BSA in the various conditions investigated. The spectroscopic estimation of the amount of protein adsorbed onto the clays (HNTs or Kao) was performed on the basis of the Amide I optical density of the FTIR non-normalized spectrum recorded before and after the rinsing to remove the excess BSA. The deconvolution procedure was applied to the Amide I band of the FTIR spectra of BSA after rinsing in order to study the conformational changes of BSA adsorbed onto the clay.

The LabVIEW program for peak fitting was based on previous works^{31, 32}. Prior to curve processing, spectra were normalized in the 1700–1600 cm⁻¹ region. This approach was adopted in order to avoid artefacts in absorptions near the limits of the region examined (1700–1600 cm⁻¹). The second derivatives of the Amide I band of the spectra examined (1700–1600 cm⁻¹ region) were then analysed in order to determine the starting data (number and position of Gaussian components) required for the deconvolution procedure. The choice of the Amide I band for structural analysis is due to the very low contribution of the amino acid side chain absorptions present in this region³³, and to its higher intensity with respect to other Amide modes. On the basis of the infrared assignment of Amide components, assuming that the extinction coefficient is the same for all the secondary structures, the secondary structure composition can be obtained from the FTIR spectra. The percentage values of the different secondary structures were estimated by expressing the amplitude value of the bands assigned to each of these structures as a fraction of the total sum of the amplitudes of the Amide I components.

The results were reproducible on different films (N=3) and on various area of the same film (N=3). The spectra recorded on N=3 area of the same film were averaged. The deconvolution was performed on the averaged spectrum. The SD bars in Figure 3, 5 and 6 refer to the data obtained on N=3 different films.

Each individual component of Amide I was assigned according to the literature ^{42, 43}, namely *ca.* 1690 cm⁻¹ (antiparallel β -sheets), *ca.* 1680 cm⁻¹ (β turns), and *ca.* 1658 cm⁻¹ (α -helix). The band in the 1626–1643 cm⁻¹ region was assigned to β -sheets, and the band at 1644-1649 cm⁻¹ was assigned to random coil (wide bandwidth) or solvated short helix (narrow bandwidth). The band at 1601–1617 cm⁻¹ was assigned to inter-molecular β -sheets.

Table S1. Results of the deconvolution procedure applied to the amide I region of the FTIR spectra of 0.5 mg/mL BSA in physiological solution with and without HNTs.

<i>BSA</i> <i>Freq. (bandwidth) cm⁻¹</i> %	<i>HNTs/BSA=0.5</i> <i>Freq. (bandwidth) cm⁻¹</i> %	<i>HNTs/BSA=4</i> <i>Freq. (bandwidth) cm⁻¹</i> %	<i>HNTs/BSA=16</i> <i>Freq. (bandwidth) cm⁻¹</i> %	<i>Assignment</i>
1603 (27) 7%	1608 (52) 26%	1604 (55) 23%	-	Inter-molecular β sheets
1618 (48) 38%	1630 (16) 7%	1637 (37) 34%	1635 (53) 69%	β sheets
1660 (56) 50%	1651 (37) 42%	1653 (24) 23%	1651 (19) 8%	α Helix
1677 (17) 1%	1681 (29) 15%	1670 (22) 19%	1668 (33) 18%	β Turns
1688 (21) 4%	1698 (17) 10%	1692 (8) 1%	1694 (17) 5%	Antiparallel β sheets

Table S2. Results of the deconvolution procedure applied to the amide I region of the FTIR spectra of 0.5 mg/mL BSA in physiological solution with and without Kao.

<i>BSA</i>	<i>Kao/BSA=0.5</i>	<i>Kao/BSA=4</i>	<i>Kao/BSA=16</i>	<i>Assignment</i>
<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	
%	%	%	%	
1603 (27)	1610 (53)	1612 (64)	1608 (59)	Inter-molecular β sheets
7%	19%	17%	10%	
1618 (48)	1633 (10)	1627 (23)	1629 (24)	β sheets
38%	11%	11%	24%	
1660 (56)	1655 (38)	1655 (35)	1658 (28)	α Helix
50%	45%	53%	53%	
1677 (17)	1679 (12)	1679 (14)	1680 (18)	β Turns
1%	4%	4%	11%	
1688 (21)	1695 (41)	1690 (38)	1695 (10)	Antiparallel β sheets
4%	21%	15%	2%	

Table S3. Results of the deconvolution procedure applied to the amide I region of the FTIR spectra of 5 mg/mL BSA in physiological solution with and without HNTs.

<i>BSA</i>	<i>HNTs/BSA=0.5</i>	<i>HNTs/BSA=4</i>	<i>HNTs/BSA=16</i>	<i>Assignment</i>
<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	
%	%	%	%	
	1610 (62)			Inter-molecular β sheets
	31%			
1638 (54)	1627 (25)	1624 (35)	1631 (48)	β sheets
54%	5%	51% s	48%	
1658(28)	1651 (44)	1660 (45)	1660 (52)	α Helix
23%	45%	47%	52%	
1679 (21)	1680 (11)	1670 (11)		β Turns
7%	1%	2%		
1690 (51)	1691 (60)			Antiparallel β sheets
16%	18%			

Table S4. Results of the deconvolution procedure applied to the amide I region of the FTIR spectra of 5 mg/mL in physiological solution BSA with and without Kao.

<i>BSA</i>	<i>Kao/BSA=0.5</i>	<i>Kao/BSA=4</i>	<i>Kao/BSA=16</i>	<i>Assignment</i>
<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	
<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	
			1620 (27) 12%	Inter-molecular β sheets
1638 (54) 54%		1638 (64) 34% s	1631 (14) 10%	β sheets
	1647 (52) 63%			Random coil
1658(28) 23%	1657 (21) 13%	1660 (48) 51%	1654 (47) 70%	α Helix
1679 (21) 7%	1685 (31) 22%	1684 (17) 12%	1681 (12) 5%	β Turns
1690 (51) 16%	1692 (6) 2%	1691 (9) 3%	1692 (10) 3%	Antiparallel β sheets

Table S5. Results of the deconvolution procedure applied to the amide I region of the FTIR spectra of 0.5 mg/mL BSA in bidistilled water with and without HNTs.

<i>BSA</i> <i>Freq. (bandwidth) cm⁻¹</i> %	<i>HNTs/BSA=0.5</i> <i>Freq. (bandwidth) cm⁻¹</i> %	<i>HNTs/BSA=4</i> <i>Freq. (bandwidth) cm⁻¹</i> %	<i>HNTs/BSA=16</i> <i>Freq. (bandwidth) cm⁻¹</i> %	<i>Assignment</i>
1604 (39) 13%	1619 (17) 18%			Inter-molecular β sheets
	1638 (10) 19%	1633 (39) 49%	1634 (38) 44%	β sheets
1644 (46) 46%				Random Coil
1658 (22) 19%	1654 (35) 42%	1652 (23) 26%	1653 (22) 20%	α Helix
1678 (25) 17%	1679 (17) 10%	1669 (21) 23%	1671 (28) 26%	β Turns
1697 (20) 5%	1694 (19) 11%	1693 (6) 2%	1694 (25) 10%	Antiparallel β sheets

Table S6. Results of the deconvolution procedure applied to the amide I region of the FTIR spectra of 0.5 mg/mL BSA in bidistilled water with and without Kao.

<i>BSA</i>	<i>Kao/BSA=0.5</i>	<i>Kao/BSA=4</i>	<i>Kao/BSA=16</i>	<i>Assignment</i>
<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	
<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	
1604 (39) 13%	1604 (13) 5%		1612 (32) 17%	Inter-molecular β sheets
	1624 (18) 8%	1623 (34) 21%	1636 (32) 27%	β sheets
1644 (46) 46				Random coil
1658 (22) 19%	1653 (42) 78%	1650 (31) 49%	1660 (35) 41%	α Helix
1678 (25) 17%	1683 (19) 7%	1678 (19) 8%	1683 (22) 15%	β Turns
1697 (20) 5%	1695 (7) 2%	1696 (43) 22%		Antiparallel β sheets

Table S7. Results of the deconvolution procedure applied to the amide I region of the FTIR spectra of 5 mg/mL BSA in bidistilled water with and without HNTs.

<i>BSA</i>	<i>HNTs/BSA=0.5</i>	<i>HNTs/BSA=4</i>	<i>HNTs/BSA=16</i>	<i>Assignment</i>
<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	
%	%	%	%	
1635 (50) 36%	1634 (43) 55%	1639 (91) 61% s	1629 (65) 50%	β sheets
1658 (39) 49%	1660 (25) 28%	1650 (30) 31%	1657 (49) 35%	α Helix
1681 (25) 4%	1678 (20) 12%	1682 (12) 8%	1675 (10) 4%	β Turns
1690 (34) 11%	1695 (17) 5%		1688 (20) 11	Antiparallel β sheets

Table S8. Results of the deconvolution procedure applied to the amide I region of the FTIR spectra of 5 mg/mL in bidistilled water BSA with and without Kao.

<i>BSA</i>	<i>Kao/BSA=0.5</i>	<i>Kao/BSA=4</i>	<i>Kao/BSA=16</i>	<i>Assignment</i>
<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	<i>Freq. (bandwidth) cm⁻¹</i>	
%	%	%	%	
	1612 (20) 5%	1613 (13) 1%	1609 (37) 15%	Inter-molecular β sheets
1635 (50) 36%	1636 (36) 37	1639 (42) 53% s	1630 (25) 17%	β sheets
1658 (39) 49%	1658 (28) 31%	1662 (28) 28%	1652 (55) 66%	α Helix
1681 (25) 4%	1682 (48) 27%	1677 (17) 14%	1681 (6) 2%	β Turns
1690 (34) 11%		1691 (8) 4%		Antiparallel β sheets