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Modulation of amyloid fibrillation of bovine β-lactoglobulin by selective methionine oxidation

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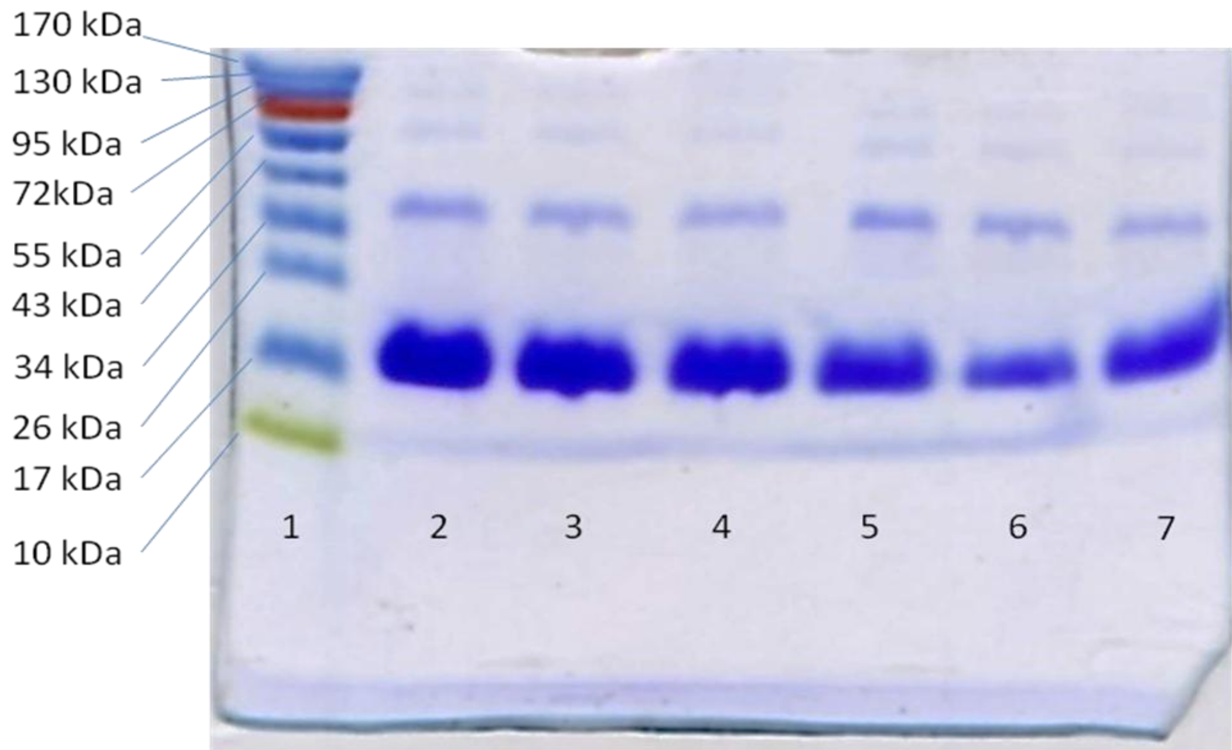

Figure S1. Represents SDS-PAGE (12%) patterns of ox-β-lg (lane 2,3,4) and native β-lg (lane 5,6,7). Lane 1 represents protein marker (Page Ruler,TM,Prestained Protein Ladder, 10,17,26,34,43,55,72,95,130 and 170 kDa respectively; Fermentus Life Science, # SM0671). Both the sample (β-lg and ox-β-lg) concentrations were kept at 108 mM .

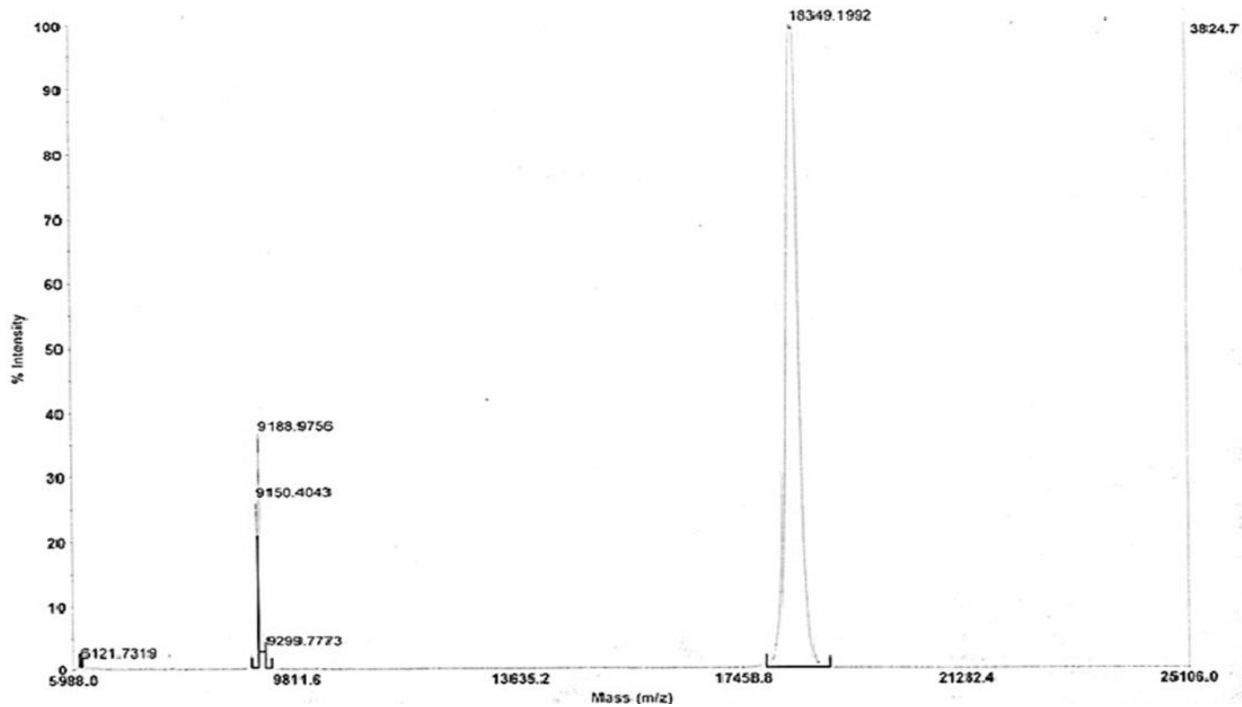

Figure S2A.

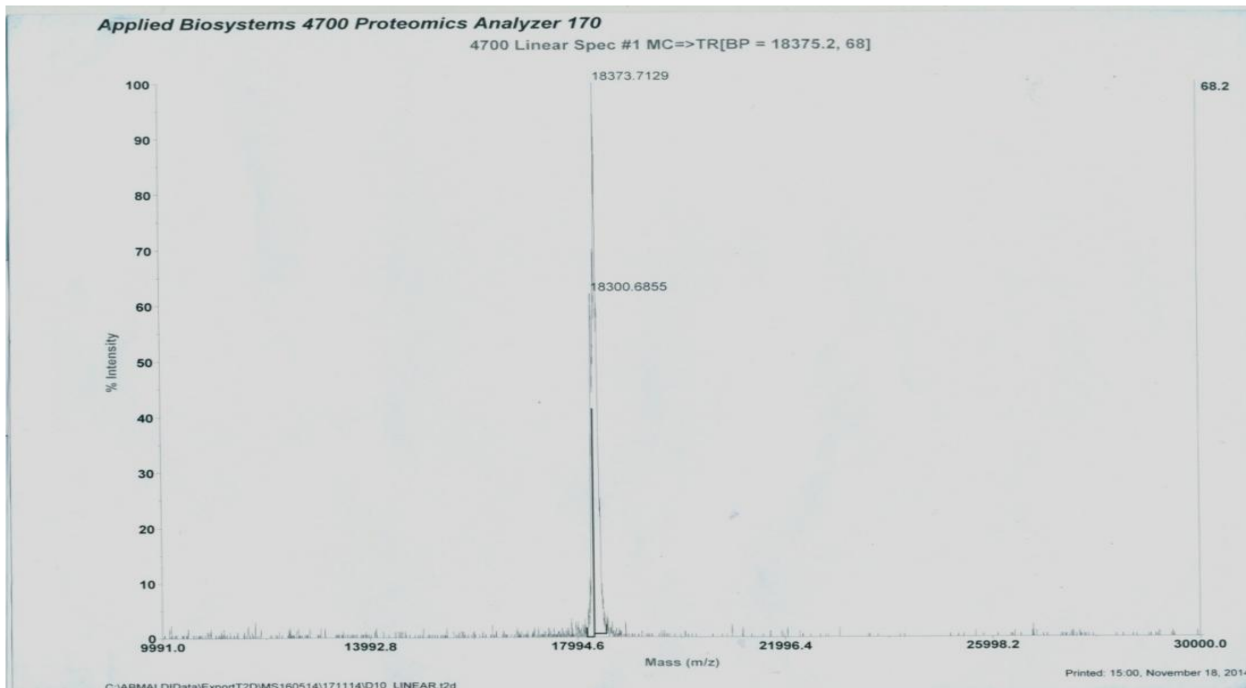

Figure S2B.

Figure S2. MALDI spectrum of (A) oxidised *β*-Lg (ox-*β*-Lg), and (B) native *β*-Lg, prepared with 50% (v/v) acetonitrile (ACN) in 0.1% (v/v) trifluoroacetic acid (TFA) and 10 mM phosphate buffer at pH 7.4.

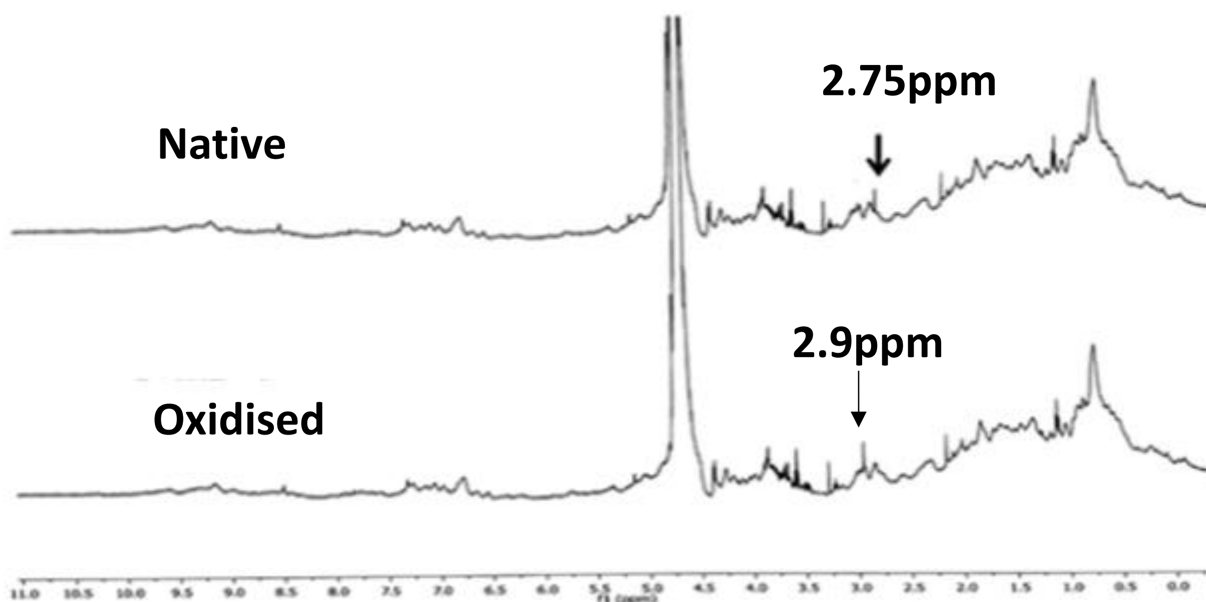

Figure S3. represents 1D ^{1}H NMR spectra of native and oxidized β-lg (100 μM) in deuterated phosphate buffer (50 mM, pD7.4) in D_2O recorded at 25 °C.

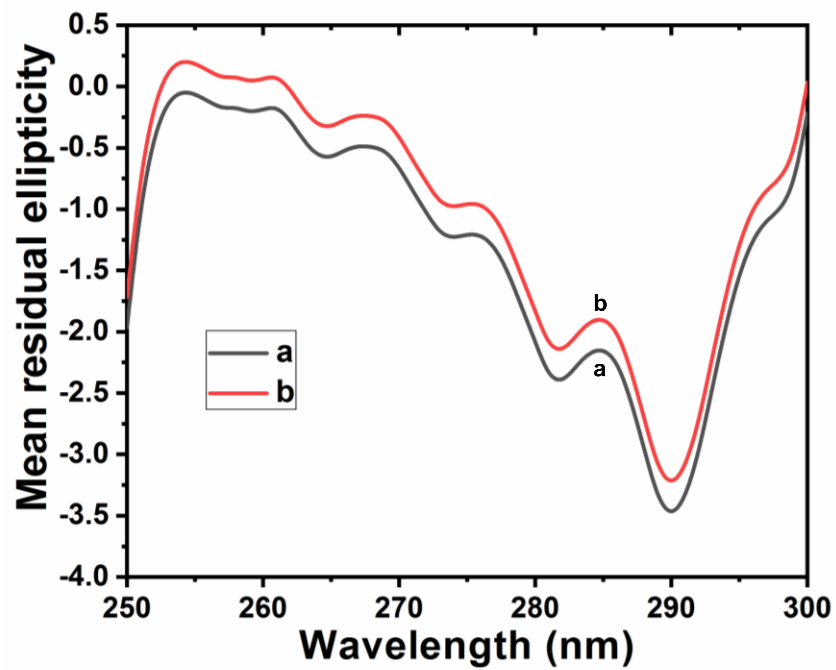

Figure S4. Near-UV CD spectrum of native *β*-Lg (profile a) and ox-*β*-Lg (profile b) in 10 mM phosphate buffer at pH 7.4 at room temperature. Both the sample concentrations were kept at 54.3 μM.

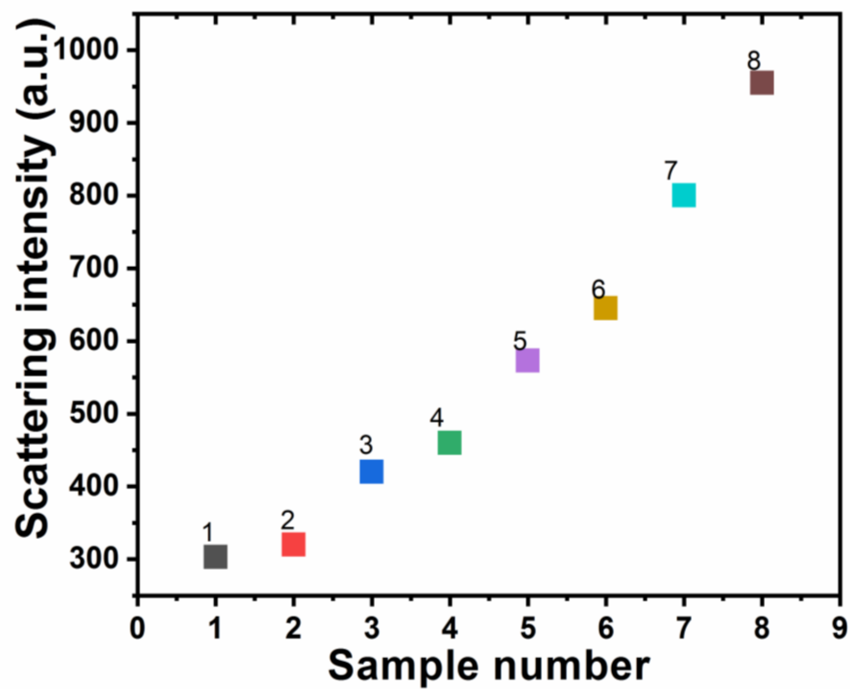

Figure S5. Rayleigh Light Scattering data (turbidity measurements) of native β-Lg (1) and ox-β-Lg (3) and incubated β-Lg and ox-β-Lg for 2 h at 37 °C (2, 4) 65 °C (5, 6) and 80 °C (7,8) respectively. The samples were excited and emitted at 350 nm.

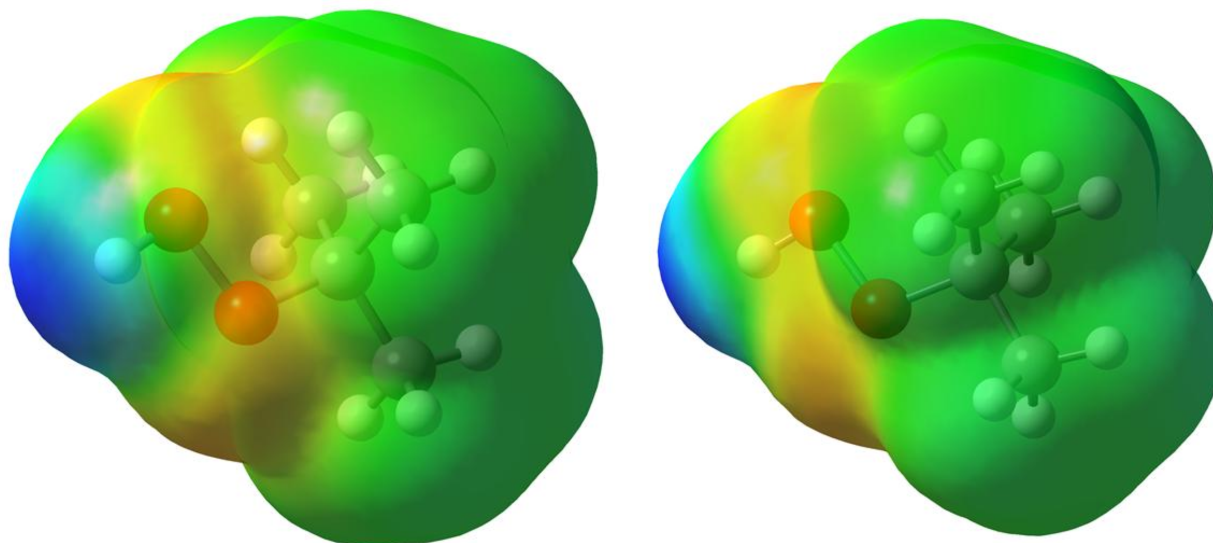

Figure S6. Molecular electrostatic potential map of *t*BHP.

Experimental

Methods

Mass spectrometric analysis

To confirm the oxidation of methionine residues of β-lg, mass spectrometric analysis was performed on tBHP-treated β-lg as the oxidizedβ-lg has higher mass. Mass spectra were acquired on an Ultra flex III MALDI-TOF/TOF mass spectrometer (BrukerDaltonics) in reflector mode using Flex Control (BrukerDaltonics). The ox-β-lg samples were prepared with 50% (v/v) acetonitrile (ACN) in 0.1% (v/v) trifluoroacetic acid (TFA). The concentrations of all the samples were 1μg/1μL.

NMR spectroscopy

Native and ox-β-lg (108 μM) were dissolved in 50 mM deuterated phosphate buffer (pH 7.4 in D_2O). Spectra were acquired at 25 °C at 600 MHz on a Varian 600 Inova NMR spectrometer. Spectra were processed and analyzed using the Sparky 3.106 software package (Freeware).

Intrinsic fluorescence

All intrinsic and extrinsic fluorescence measurements were performed on a Shimadzu spectrofluorimeter (Shimadzu 5301 PC) at 25 °C by using 1 cm quartz cuvette. Intrinsic fluorescence experiments were performed with protein concentration at 13.6μM. To monitor the intrinsic fluorescence of β-lg, protein samples were excited at 295 nm and the emission spectra were recorded between 310 nm and 400 nm. The excitation and emission slits were set at 3 nm and 5 nm respectively for all fluorescence measurements. All the experiments were performed at least three times.

8-anilinonaphthalene-1-sulfonic acid (ANS) titration study

ANS titration assays were performed by adding small aliquots of concentrated ANS solution to protein solution. Oxidized β-lg (ox-β-lg) and β-lg concentrations were about 13.6 μM and molar ratio [ANS]/ [protein] was varied in the range 0.1 - 2.8. Dilutions on ANS never exceeded 5% of the starting volume. Protein samples were excited at 370 nm and emission was measured at 510 nm.

Table S1: Structural integrities of native (*β*-Lg and ox-*β*-Lg) and incubated (*β*-Lg and ox-*β*-Lg for 4 h at 37 °C, 65 °C and 80 °C) samples were determined by CDNN 2.1 software.

Samples	% of α-helix	% of *β*-sheet	% of *β*-turn	% of Random coil
Native *β*-lg	28.7	18.8	19.8	32.7
Ox-*β*-lg	30.6	17.7	18.8	33
β-lg (incubated at 37 °C)	30.4	19	18.6	32.1
Ox-*β*-lg (incubated 37 °C)	28.7	19.78	17.7	33.8
β-lg (incubated at 65 °C)	19.3	25.16	18.8	36.9
Ox-*β*-lg (incubated at 65 °C)	17.16	26.19	18.51	38.2
β-lg (incubated at 80 °C)	14.23	28.04	18.37	39.34
Ox-*β*-lg (incubated at 80 °C)	13.6	28.3	18.12	39.9