

Effect of Temperature On Re-entrant Condensation of Globular Protein in Presence of Tri-Valent Ions

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Research Article

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Abstract

Globular proteins play several essential roles in functioning different mechanisms of the living organisms, and the stability of such protein molecules in an aqueous solution is strongly affected by multivalent ions. In this article, we have systematically studied the effect of temperature (between 5 and 25°C) on the re-entrant condensation behaviour of bovine serum albumin (BSA) in the presence of trivalent ions, Yttrium (Y^{3+}), and Lanthanum (La^{3+}). The effect of temperature is explained considering the optical properties of the protein, i.e., from the optical absorption and emission behaviours. The absorption in the visible region and the fluorescence emission of BSA becomes maximum at the lowest temperature. The decrement of mobility at lower temperature is responsible for fluorescence enhancement. Moreover, the activation energy of the turbid or viscous phase of the BSA protein under re-entrant condensation is enhanced in comparison with the transparent phase and the corresponding energy value is estimated from the fluorescence study.

Introduction

Proteins, an important biomolecule, play a major role in the different mechanisms of a living system. The interaction among protein molecules in solution and their stability is the fundamental aspect of many biochemical processes [1, 2]. Globular protein is very much important among other classes of proteins and plays a crucial role as messenger and transporter [3, 4]. These proteins possess both positive and negative surface charges, and the net surface charge depends on its isoelectric point (pI) [5, 6]. Therefore, at different physicochemical environments created by dissolved ions (both cations and anions), polyelectrolytes, salt concentration, ionic strength, temperature, pH and solvent, the protein molecules exhibit various interactions and complex phase behaviours [7- 11]. Different types of interactions, such as hydrophobic-hydrophilic interaction, electrostatic interaction, hydrogen bond formation, and van der Waals interaction, are mainly responsible for the rich phase behaviours of proteins in solution [12-14]. The phase behaviour of proteins in solution includes protein crystallization [15], protein condensation [16], protein aggregation [17], liquid-liquid phase separation (LLPS) [18, 19], and re-entrant condensation (RC) [20-25]. The study on the phase behaviour of proteins and the related interaction plays a key role in proper understanding of the protein misfolding and related physiological diseases [26, 27].

Re-entrant condensation is a fascinating phase behaviour observed in several globular proteins, DNA, polyelectrolytes, and charged colloids [24, 28-30]. Globular proteins such as bovine serum albumin (BSA), human serum albumin (HSA), ovalbumin, and β -lactoglobulin (β -LG) exhibit a condensation effect in the presence of different multivalent salts such as yttrium chloride (YCl_3), lanthanum chloride ($LaCl_3$), iron chloride ($FeCl_3$), and aluminium chloride ($AlCl_3$) for their particular concentration region [31-35]. Within two critical cation concentrations, C_1 and C_2 ($C_1 < C_2$), the protein solution appears as turbid rather than the usual transparent solution [24]. Similar to tri-valent ions, the presence of tetra-valent ion zirconium chloride ($ZrCl_4$) can create re-entrant condensation of BSA [36]. The condensation can be influenced by the solvent, type of ions, ionic strength of the solution, etc [21, 25]. Compared to the neutral

salts such as YCl_3 or $LaCl_3$, a narrow re-entrant phase occurs for acidic salts such as $AlCl_3$ and $FeCl_3$ [20]. The presence of monovalent salt ($NaCl$) influences the re-entrant behaviours of HSA molecules in the presence of YCl_3 and shifts the phase boundary towards higher salt concentration [37]. Like cations, anions also influence the protein-protein interaction and lead to re-entrant condensation [21]. Effective interaction of BSA under the re-entrant phase transition in the presence of $LaCl_3$ is enhanced significantly when the Cl^- was replaced by NO_3^- ions. Short-range and long-range electrostatic interactions and the entropic contribution play a crucial role in condensation [24]. Along with this, the charge inversion of proteins due to the binding of trivalent ions to the protein carboxyl groups is also considered as a reason behind this re-entrant condensation [31].

Temperature is a crucial parameter for proteins in solution, and protein-protein interaction is highly affected by the temperature [7, 38]. A protein aggregated structure forms at a lower temperature (5-10°C) in the condensed phase under re-entrant condensation of β -LG in the presence of YCl_3 [17]. Globular protein, β -LG, in the presence of YCl_3 , shows a lower critical solution temperature (LCST) phase behaviour at lower salt concentration and an upper critical solution temperature (UCST) phase behaviour at higher salt concentration. At the same time, BSA in the presence of the same salt exhibits LCST type phase behaviour only at lower salt concentration [38]. This phase behaviour of BSA at a lower salt concentration in the presence of trivalent ions is due to the cation-protein binding and bridging between protein molecules [7]. Moreover, BSA in the presence of YCl_3 exhibits temperature-dependent LLPS phase behaviour [39]. Above 55°C, the phase transition of protein is irreversible, and below that temperature, it shows a temperature-dependent reversible growth behaviour. Globular protein exhibits fluorescence property due to the presence of tryptophan and tyrosine amino acid residues. Protein-protein interaction can also be explained by studying the fluorescence properties of the proteins [40, 41]. In our previous work, we observed the enhancement of fluorescence emission in the re-entrant region, and it was correlated with the hydrodynamic size as well as mobility of the protein [42]. Though a number of literatures are available on different aspects of the re-entrant phase behaviour of globular proteins and related interactions, the effect of temperature on the fluorescence emission under the re-entrant condensation is still not explored properly.

In this article, we have investigated the optical absorption and also the emission behaviours of globular protein BSA in the presence of trivalent salts (YCl_3 and $LaCl_3$) with the variation of temperature from 5 to 25°C. With the increment of temperature, the protein solution becomes more fluidic and as a result the fluorescence quenching enhances. Protein undergoes re-entrant condensation in the presence of trivalent ions and the solution becomes turbid and more viscous, and accordingly the fluorescence behaviour is also changed. The activation energy value of the turbid phase is estimated from the fluorescence study and is compared with the transparent phase of the protein as obtained at lower and higher salt concentrations.

Experimental Details

Bovine serum albumin (BSA) (product no. A2153), yttrium chloride (YCl_3) (Product No. 464317), lanthanum chloride (LaCl_3) (product no. 31820) were purchased from Sigma-Aldrich and were used as received. The distilled water of resistivity $\approx 18.2 \text{ M}\Omega\text{-cm}$ from the Milli-Q system from Millipore was used throughout the experiments. BSA solutions of 25mg/ml were prepared in Milli-Q water. Tri-valent ions (Y^{3+} and La^{3+}) were dissolved in the protein solutions to observe the re-entrant condensation, and salt concentrations were varied from 0 to 20mM. No buffer solution was used to avoid the effect of other co-ions if any. The temperature effect on BSA in the presence of trivalent ions and related fluorescence behaviour was studied in the temperature range of 5-25°C. UV-vis absorption spectra were recorded using a Shimadzu UV-1800 UV-vis spectrophotometer in the range of 200-700 nm for three different solution temperatures of 5, 15, and 25°C. Fluorescence emission spectra were taken in the above-mentioned temperature region with a 5°C interval, using JASCO FP-8500 fluorescence spectrometer. A Peltier thermostat-controlled (water-cooled) temperature controller ETC-815 associated with the fluorescence spectrophotometer was utilized to control the temperature. The spectra were taken in the range of 280-600 nm with a quartz cell of 10 mm path length. Both excitation and emission slit widths were set at 5 nm.

Results And Discussion

Re-entrant condensation was observed upon mixing the tri-valent salts (YCl_3 and LaCl_3) with BSA (see the supplementary file) [42]. Upon mixing the salts with protein, a turbid-like solution was observed within a certain range of salt concentration, and below and above that salt concentration protein solution becomes transparent. For Y^{3+} , the protein turbidity was observed around 3-5 mM salt concentration, whereas, for La^{3+} , it was observed at around 1-3 mM salt concentration. The UV-vis absorption spectrum of BSA at room temperature (25°C) is shown in Fig. S2. BSA shows absorption at around 278 nm [42]. Upon mixing the tri-valent salts (Y^{3+} and La^{3+}), the absorption of BSA is not affected, but the absorption in the optical region, i.e., at around $\lambda \approx 630 \text{ nm}$, is affected. The variation of optical absorption is shown in Fig. 1. Fig. 1(a) and (b) corresponds to the variation of the optical absorption of BSA at a wavelength of $\lambda \approx 630 \text{ nm}$, and for the three different temperatures of 5, 15, and 25°C in the presence of 0-20 mM yttrium and lanthanum, respectively. From the figure, it is clear that in the re-entrant region, i.e., when protein solution becomes turbid, the optical absorbance becomes maximum. The rate of enhancement is also increased for lowering the solution temperature.

The fluorescence emission of BSA at room temperature (25°C) is also shown in Fig. S2. BSA shows an emission at around 345 nm for an excitation at 278 nm. The variation of the maximum emission intensity, i.e., emission at 345 nm, at room temperature (25°C), in the presence of yttrium (Y^{3+}) ions is shown in Fig. 2(a). The enhancement of emission intensity is observed in the re-entrant region. The emission intensity of the same samples was recorded for other temperatures also, and the variation of the maximum emission intensity is also plotted in the same figure.

Like yttrium, the fluorescence emission of BSA in the presence of La^{3+} was also observed. The maximum emission intensity of BSA in the presence of La^{3+} , for different temperatures, is shown in Fig. 2(b). From both the figures, it can be observed that the emission intensity becomes maximum in the re-entrant phase region. Along with this, it is observed that the emission intensity is always higher for lower temperature. This can be further understood from Fig. 3. In this figure, the maximum emission intensity of BSA with temperature variation is plotted for a particular salt concentration. Fig. 3(a) and 3(b) corresponds to the variation of emission intensity of BSA for different temperatures, in the presence of Y^{3+} and La^{3+} , respectively. Though the intensity variation is less but the decrement of the maximum emission intensity with increasing temperature is clearly visible.

The temperature dependence of the fluorescence emission can be utilized for the measurement of the activation energy (E_a) of the protein within the specific temperature range [43]. The activation energy is associated with the interconversion between the fluorescent and non-fluorescent state of the protein [44]. The activation energy can be calculated from the Arrhenius equation, which can be written as [44, 45]:

$$\ln \left(\frac{F_0}{F} \right) = A - \frac{E_a}{RT} \dots (1)$$

where, F_0 is maximum fluorescence intensity at lowest temperature and F is the maximum fluorescence intensity at other temperatures. A is exponential factor, R is the universal gas constant, and T is the absolute temperature. Using the equation, the variation of emission intensity with the reciprocal of temperature ($1/T$) is plotted in Fig. 4. The Arrhenius plot of BSA in the presence of various concentrations of yttrium and lanthanum ions are plotted in Fig. 4(a) and 4(b), respectively.

From the slope of the linear fitting of each plot, the activation energy corresponding to the particular samples are calculated and tabulated in Table 1. The variation of E_a of the BSA protein in the presence of yttrium and lanthanum salts of different concentrations are plotted in Fig. 5(a) and 5(b) respectively.

Table 1

The values of activation energy calculated from Arrhenius plot for BSA in the presence of yttrium (Y^{3+}) and lanthanum (La^{3+}) ions.

Yttrium (Y^{3+}) concentration (mM)	Activation energy (E_a) with error (kJ mol^{-1})	Lanthanum (La^{3+}) concentration (mM)	Activation energy (E_a) with error (kJ mol^{-1})
0	3.48 ± 0.612	0	3.48 ± 0.612
1	4.17 ± 0.439	1	3.53 ± 0.210
3	5.15 ± 0.457	3	3.98 ± 0.251
5	5.14 ± 0.437	5	7.21 ± 0.774
7	3.56 ± 0.690	7	6.61 ± 1.503
10	3.87 ± 0.707	10	4.23 ± 0.785
20	1.75 ± 0.235	20	4.26 ± 0.220

From the analysis, it is thus clear that in the presence of both the salts, the activation energy of BSA protein becomes maximum in the re-entrant region, and takes the values of ≈ 5.1 and 7.2 kJ mol^{-1} for Y^{3+} and La^{3+} ions respectively. The activation energy associated with the fluorescence of pure BSA in the above-mentioned temperature range is obtained as $\approx 3.48 \text{ kJ mol}^{-1}$. The activation energy as obtained is associated with the delicate micro-structural modifications around the tryptophan residues with the variation of surrounding physical or chemical environments. When the temperature of the protein solution is reduced, the fluidity of the solution also decreases [43], and due to the less mobility of the constituent molecules, the enhancement in the fluorescence emission is observed. On the other hand, due to the increment of temperature, the rotations and vibrations among the fluorophores may enhance and as a result the non-radiative decay increases and fluorescence intensity decreases [46]. In the presence of the trivalent ions, it was proposed that the apparent size of the constituent BSA solutes in the aqueous solution enhances and the mobility of the proteins decreases, and as a result the enhancement in the fluorescence emission is observed [42]. Thus, the effect of trivalent ions under re-entrant condition is nearly similar to the effect of reduction in temperature of the BSA solution. Thus, the activation energy as obtained at condensed phase is higher than the transparent phase of the protein solution. The occurrence of the lower mobility or higher viscosity of the solution leads to the increment of activation energy in the re-entrant region.

Conclusions

Behaviour of bovine serum albumin (BSA) is studied under the re-entrant condensation with temperature variation. BSA shows re-entrant condensation in the presence of trivalent salts yttrium chloride (YCl_3) and lanthanum chloride ($LaCl_3$), and the corresponding fluorescence emission is compared within the temperature range between 5 and 25°C . The fluorescence emission intensity of the protein becomes

maximum in the re-entrant region within the selected temperature range. Moreover, both the visible absorption and the fluorescence emission intensity become maximum at the lowest temperature (i.e., at 5°C). With the decrement of temperature, the mobility of the protein solution decreases, that is responsible for the enhancement of the fluorescence emission of BSA protein. With increase in temperature, the rotational and vibrational movements among the protein molecules increases and leads to the enhancement of the nonradiative decay and decrement of the fluorescence emission intensity. However, in the re-entrant region, due to the enhancement of mobility and apparent size of protein, the fluorescence intensity also increases. The variation in fluorescence emission intensity is utilized to calculate the activation energy of BSA at different physiochemical conditions. The results confirm that the activation energy is higher (≈ 5.1 and 7.2 kJ mol^{-1} for Y^{3+} and La^{3+} ions respectively) for the condensed phase under re-entrant condensation in comparison with the transparent phase ($\approx 3.48 \text{ kJ mol}^{-1}$) of the protein solution.

Declarations

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Author Declarations

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Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval/declarations This is an observational study. No ethical approval is required.

Consent to participate Consent was obtained from all the authors.

Consent for publication Not applicable

Availability of data and material Not applicable

Code availability Not Applicable

Author contributions SP and SK conceptualized the study and designed the experiments. SP performed all the experiments and wrote the manuscript. SK supervised the work and approved the manuscript.

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Figures

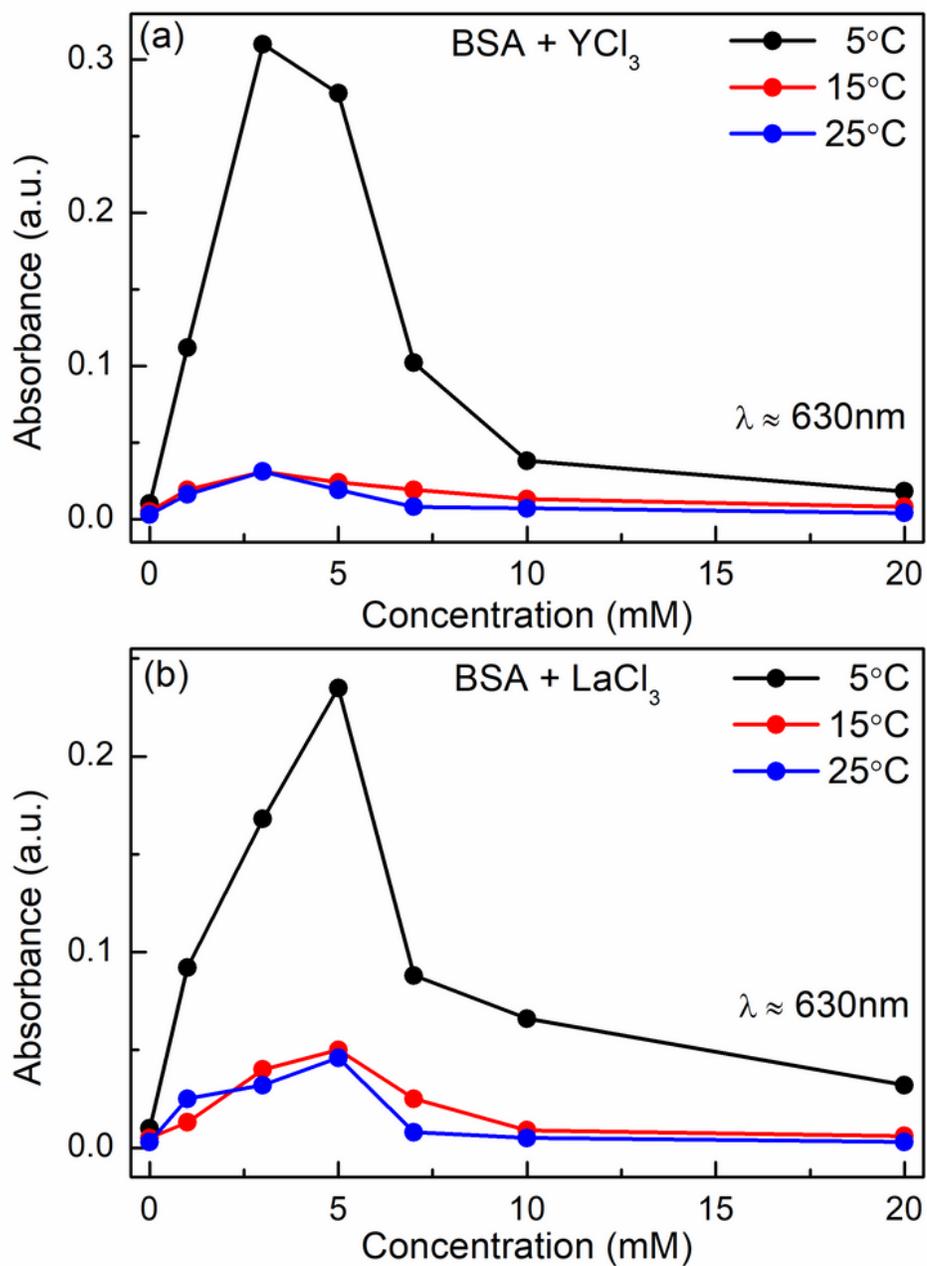


Figure 1

Variation of the optical absorption of BSA (25 mg/ml) for three different temperatures (5, 15, and 25°C) in the presence of (a) yttrium (Y^{3+}), and (b) lanthanum (La^{3+}) ions for different salt concentrations (0 to 20 mM) at a wavelength of 630 nm.

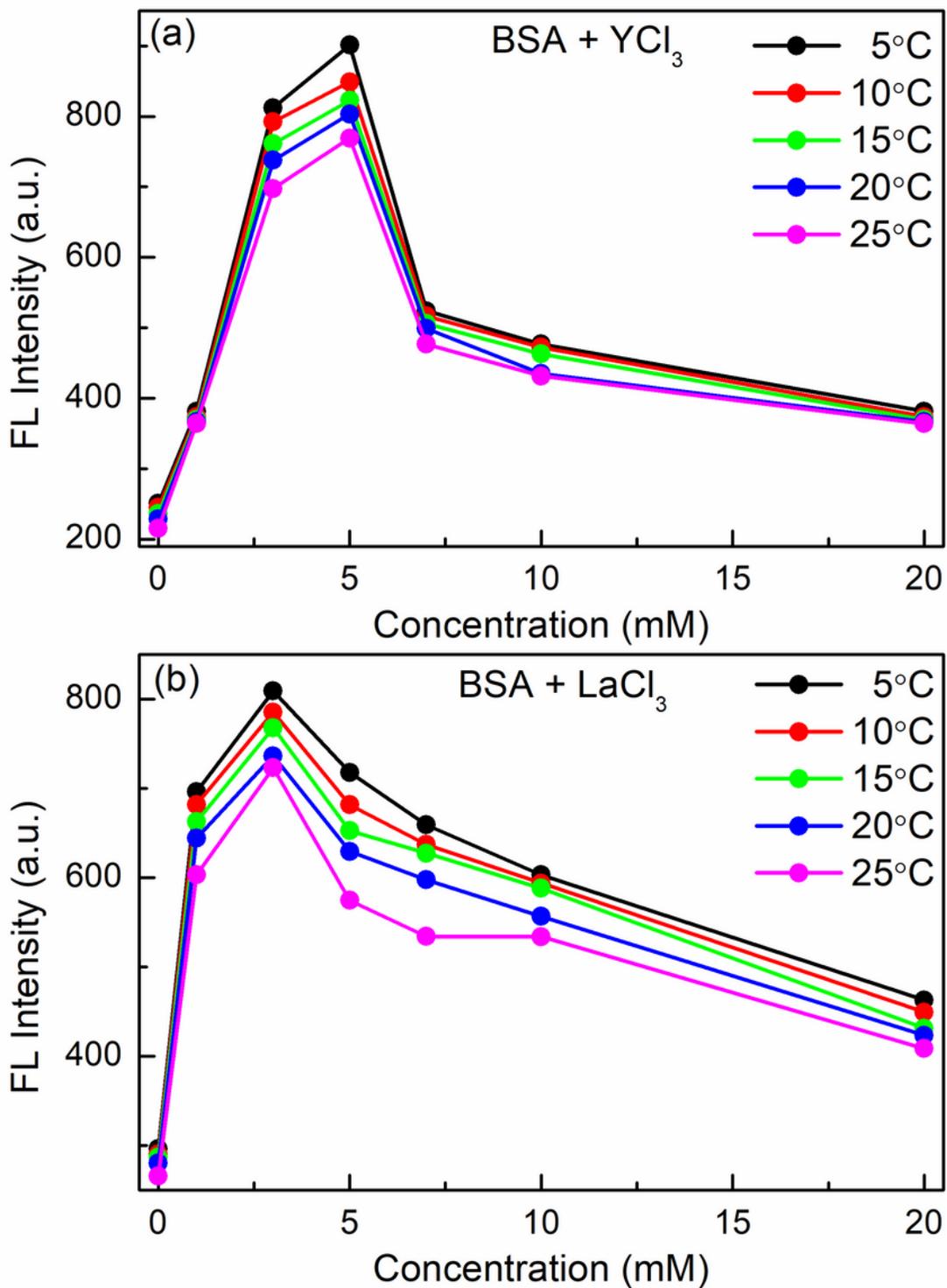


Figure 2

Variation of the maximum fluorescence emission intensity of BSA protein in the presence of (a) yttrium (Y³⁺), and (b) lanthanum (La³⁺) ions at different temperatures for salt concentration of 0-20 mM.

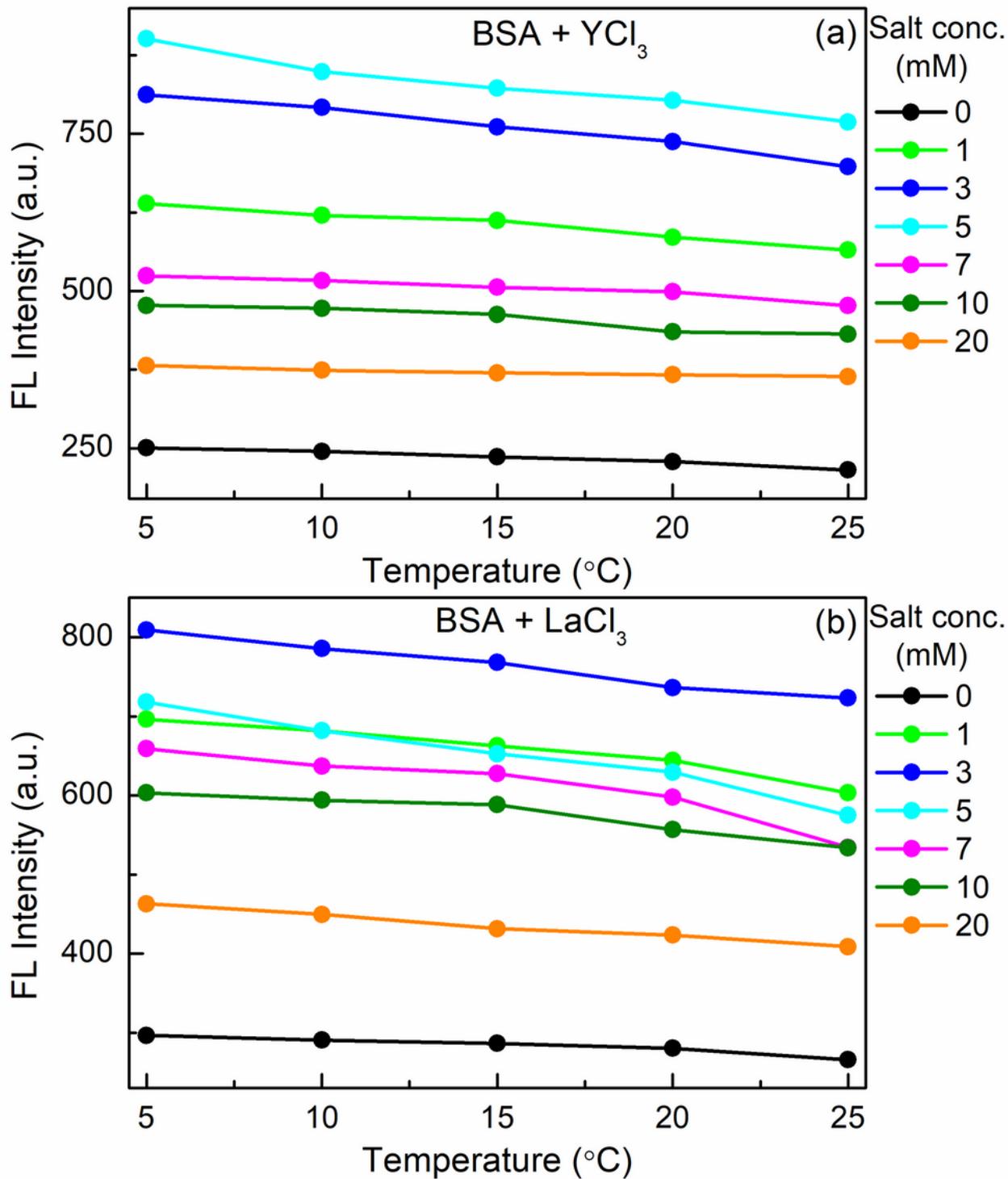


Figure 3

Variation of maximum emission intensity of BSA with temperature in the presence of (a) yttrium (Y³⁺), and (b) lanthanum (La³⁺) ions at different salt concentrations.

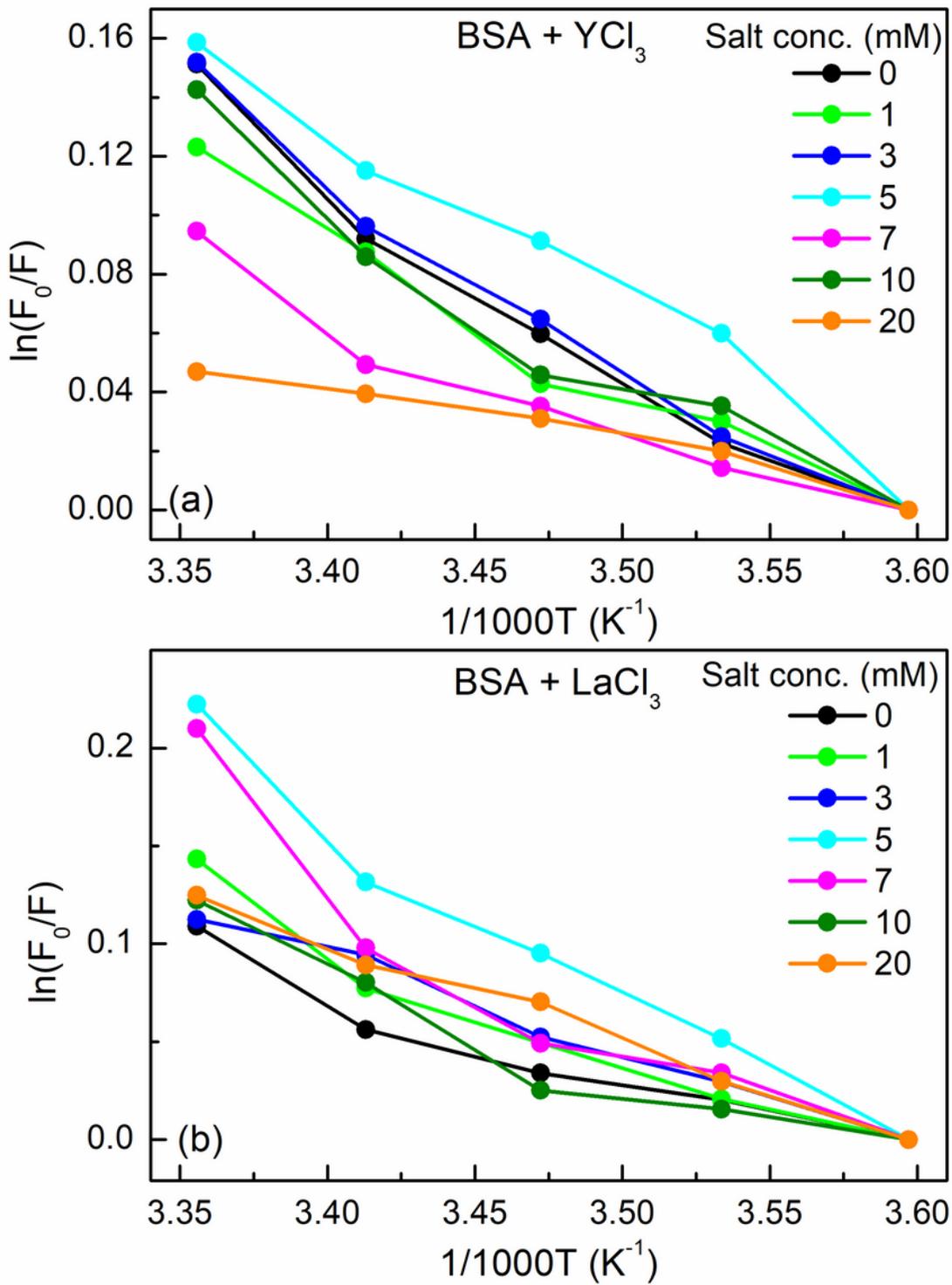


Figure 4

Arrhenius plots of BSA protein in the presence of different concentrations of (a) yttrium (Y³⁺) and (b) lanthanum (La³⁺) ions in the temperature range of 5-25°C.

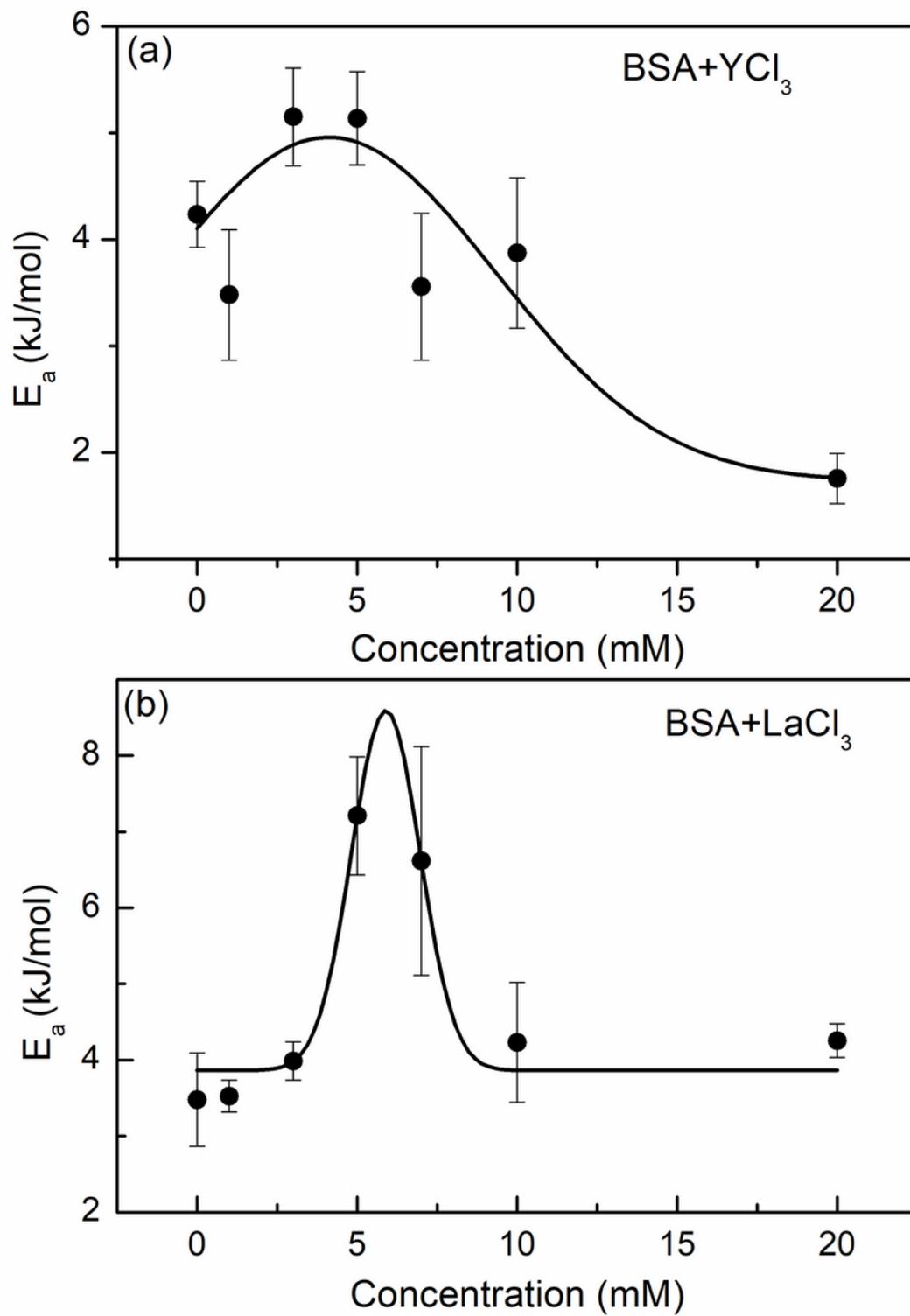


Figure 5

Variation of activation energy (E_a) of BSA protein in the presence of different concentrations of (a) yttrium (Y³⁺) and lanthanum (La³⁺) ions.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterials.docx](#)