

October 19, 2011

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### Recommended Citation

Park, Byoung Kyoo; Yi, Namwoo; Park, Jaesung; Choi, Tae Y.; Lee, Jin Young; Busnaina, Ahmed; and Kim, Dongsik, "Thermal conductivity of bovine serum albumin: a tool to probe denaturation of protein" (2011). *Mechanical and Industrial Engineering Faculty Publications*. Paper 38. <http://hdl.handle.net/2047/d20001186>

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# Thermal conductivity of bovine serum albumin: A tool to probe denaturation of protein

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(Received 7 July 2011; accepted 21 September 2011; published online 19 October 2011)

We demonstrate a strong correlation between denaturation of bovine serum albumin (BSA) and the thermal conductivity  $k$  of aqueous solutions of BSA. When denaturation of BSA began,  $k$  dropped significantly. These results suggest that  $k$ , i.e., the ability of a protein to transport passively applied thermal energy, can be exploited to probe the conformational dynamics of BSA and potentially of other proteins. The technique of protein analysis demonstrated in this work is expected to be useful in micro-total-analysis systems because it is easier to miniaturize and to integrate into a device than is conventional differential scanning calorimetry analysis. © 2011 American Institute of Physics. [doi:10.1063/1.3652704]

Proteins catalyze chemical reactions that are involved in cell signaling, signal transduction, the immune response, cell adhesion, and have structural functions.<sup>1,2</sup> They form a three-dimensional folded (tertiary) structure that achieves the lowest possible free energy of the peptide chain and the surrounding solvent.<sup>3</sup> All biological activities are thermally active, but they work only in a narrow range of temperature  $T$ . If  $T$  exceeds a certain range, proteins lose their tertiary structures, i.e., are denatured. Therefore, delineating the energy flow and thermal transport in proteins is a key to understanding how they maintain the optimal temperature in the biological system.

Many studies on denaturation of proteins have been conducted using various techniques, including fluorescent and calorimetric methods.<sup>4–14</sup> The status of bovine serum albumin (BSA) during denaturation has been analyzed and confirmed using far-ultraviolet circular dichroism.<sup>13,14</sup> Thermal analysis using differential scanning calorimetry (DSC) discloses the thermodynamic conditions under which denaturation occurs and quantifies the enthalpy of denaturation. Yu and Leitner<sup>15</sup> calculated the thermal conductivity  $k$  of two structurally distinct proteins, myoglobine and a green fluorescent protein (GFP). The calculated thermal conductivities are  $0.27 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$  in both cases. Iervolino *et al.*<sup>16</sup> measured the  $k$  of solutions of L-lysine using a calorimetric chip and estimated the  $k$  of L-lysine by extrapolating the data. No research has reported how  $k$  of proteins is affected by their denaturation state, i.e., by change in  $\text{pH}$  and  $T$ . Thermodynamic properties such as heat capacity change significantly during denaturation of a protein because this process affects the extent of hydration of its group and the proteins degrees of freedom.<sup>17</sup> Similarly,  $k$  of a protein can be expected to change significantly during denaturation.

BSA has been widely investigated because it is commonly used in clinical application and fundamental studies

of protein chemistry and molecular biology.<sup>4–8,18</sup> It has been reported that DSC studies of BSA reveal the thermodynamics of the protein associated with denaturation, specifically the change in specific heat and enthalpy near the denaturation temperature  $T_d$ .<sup>6–8</sup> However, relatively less attention has been paid to  $k$  of BSA ( $k_{BSA}$ ).<sup>18</sup> Therefore, we measured  $k$  of aqueous solutions of BSA ( $k_{sol}$ ) by varying  $T$  and  $\text{pH}$  to determine how denaturation affects thermal transport in the solutions. The measured  $k$  data were compared with the results of the DSC analysis,<sup>7</sup> with discussions on the correlation between  $k$  and the denaturation process. BSA solutions were prepared by mixing BSA powder (biotechnology grade BSA, 0903, AMRESCO) with deionized (DI) water. The BSA powder were prepared using cold alcohol isolation and were thus free of both DNase and RNase and virtually free of globulins and other interfering contaminants. The  $\text{pH}$  of the solutions was adjusted by adding HCl or NaOH.

We employed the three-omega method<sup>19–25</sup> to measure  $k_{sol}$  (Fig. 1(a)). Application of this method to thermal analysis of small-volume liquid samples has demonstrated that the method is particularly suited for measuring  $k$  of samples which are electrically conducting or as small as sub-nanoliter in volume.<sup>22–24</sup> The sensor was inserted into a copper block inside a test chamber (Fig. 1(b)). A water bath was used to control the  $T$  of the chamber to within  $\pm 0.1^\circ\text{C}$ . The experimental setup is similar to those used for measuring  $k$  of small-volume liquid samples and solid thin films.<sup>22–25</sup> All measurements were repeated 10 times for each sample to assess the random error. The accuracy of measurement was tested by measuring DI water, ethylene glycol, and methanol; the deviations from reference values<sup>26</sup> were all  $<3\%$ . The maximum standard deviation (sd) was 2.24% and the average sd was 1.29%.

At  $25^\circ\text{C}$  and  $\text{pH}$  7,  $k_{sol}$  decreased as BSA concentration increased (Fig. 2). At a relatively low concentration of 1 mg/ml (0.1 wt. %, 14.4 mM),  $k_{sol}$  was close to  $k_{DI}$ , (measured  $k$  of DI water,  $0.608 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$ ). Simple linear extrapolation of the data yields the  $k_{BSA}$   $0.265 \pm 0.08 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$ . This

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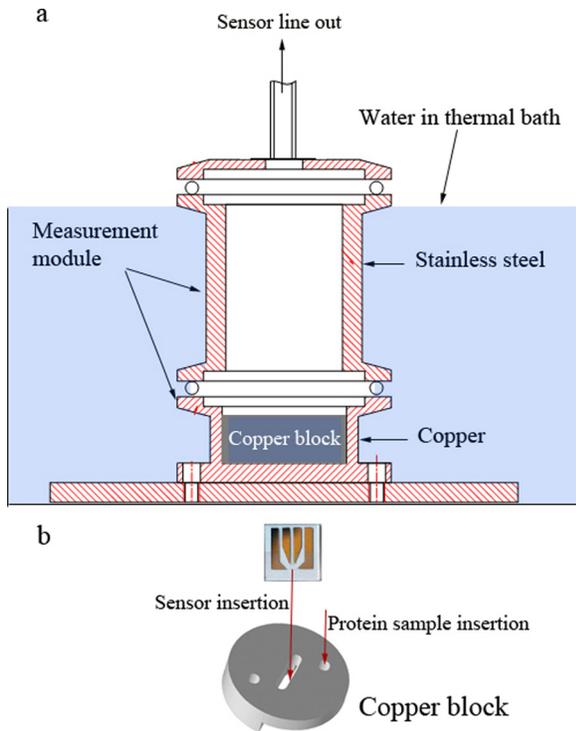


FIG. 1. (Color online) Experimental setup: (a) measurement module and (b) three-omega sensor and copper block.

estimated value is close to numerical predictions of  $k$  for myoglobin and GFP ( $\sim 0.27 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$ ).<sup>15</sup>

The  $k$  of 5 wt. % BSA solutions was measured with as a function of  $T$  and  $pH$  (Fig. 3(a)). Because the structure of a protein is known to be influenced by the acidity of the buffer solution as well as by  $T$ ,<sup>7</sup> samples were prepared with  $5.05 \leq pH \leq 10.05$  and measurements were conducted at  $25^\circ\text{C} \leq T \leq 85^\circ\text{C}$  in increments of  $10^\circ\text{C}$ . However, at  $pH = 5.05, 8.20,$  and  $10.05$ , measurements at  $85^\circ\text{C}$  were not possible because the denatured proteins adhered to the chamber and sensor surfaces. The maximum sd was 3.68% and the average sd was 1.48%. The main source of this variability may be sample inhomogeneity, which resulted in variations in agglomeration and settlement characteristics. At fixed  $pH$ ,  $k$  of each BSA solution increased with the same slope until the  $T$  reached a critical point. The slope was approximately equal to the temperature coefficient of  $k_{DI}$ , which increases from  $0.608 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$  at  $25^\circ\text{C}$  to  $0.674 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$  at  $85^\circ\text{C}$ . When  $T$  was further increased above a certain critical value  $T_i$ , the slope of each  $k$  curve deviated from that of DI water. In this work,  $T_i$  was defined as the temperature at which  $\Delta k = k_{sol} - k_{DI} - \Delta k'$

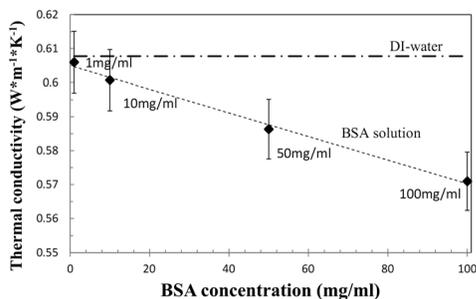


FIG. 2.  $k_{sol}$  with various concentrations.

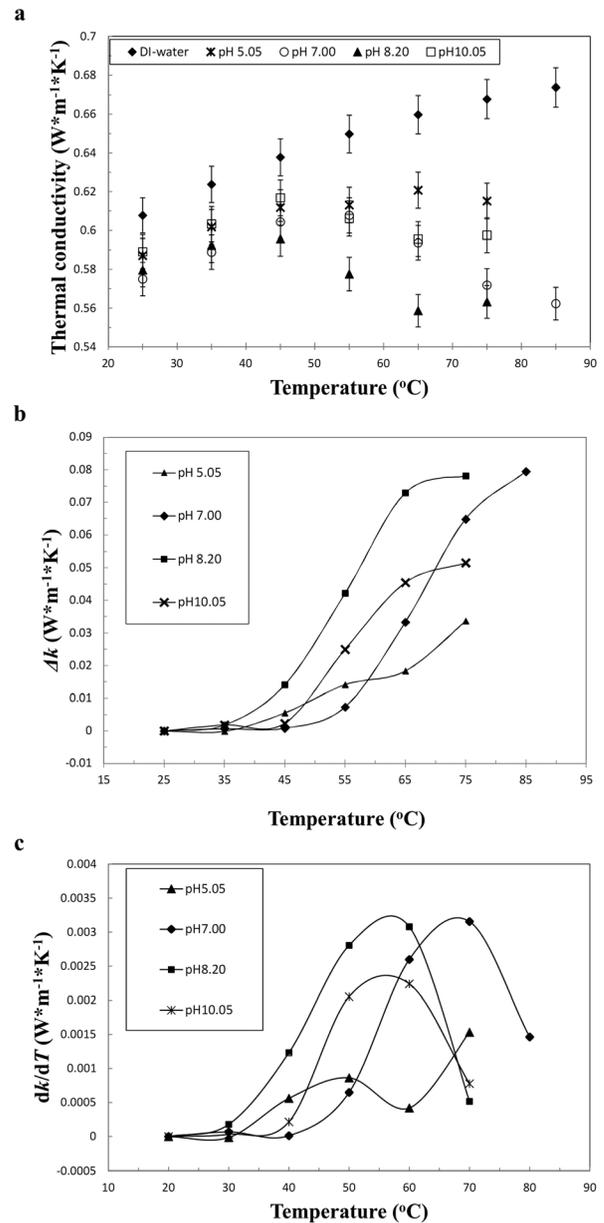


FIG. 3. (a) Thermal denaturation effect on  $k_{sol}$ , (b)  $k$  difference between base fluid and BSA solution (after cancelling out the difference at  $25^\circ\text{C}$  for each curve), and (c) Rate of change of  $k$  difference during denaturation process.

$> 0.005 \text{ W}\cdot\text{m}^{-1}\cdot\text{K}^{-1}$  ( $\Delta k'$ : the difference at  $25^\circ\text{C}$  for each curve). Contrary to the tendency of base fluids,  $k$  of protein solution decreased when  $T > T_i$ . Accordingly,  $T_i$  corresponds to the onset of denaturation.

The variation of  $\Delta k$  with  $T$  (Fig. 3(b)) was not due the temperature-dependence of  $k_{DI}$ , but corresponds to the change in the thermal characteristics of BSA. It is noted that the effect of HCl and NaOH on  $k$  is almost independent of  $T$  and is negligible because the chemicals were added at miniscule quantities ( $< 0.02 \text{ wt. } \%$ ). Subtracting  $k_{DI}$  and  $\Delta k'$  for each  $pH$  curve leaves only the effect of protein denaturation on  $k_{sol}$ . At  $T < T_i$ ,  $\Delta k = 0$  regardless of  $pH$ , but began to increase at  $T_i$  and saturated at a temperature  $T_s$ . Saturation of  $\Delta k$  indicates that the change in  $k_{sol}$  by denaturation ceases once it is fully unfolded. Assuming that  $k_{sol}$  is proportional to the degree to which each protein that has unfolded,  $\Delta k$  at a certain

TABLE I. Comparison of temperatures  $T_i$  at which BSA denaturation begins and  $T_d$  at which it is maximal, with DSC study.<sup>7</sup>

pH	Measurement		pH	DSC study <sup>7</sup>	
	$T_i$ ( $\pm 5$ °C)	$T_d$ (°C)		$T_i$ (°C)	$T_d$ (°C)
5.05	44.0	50.0, 70.0	4.99	41.6	59.8
7.00	53.0	68.0	7.00	51.9	64.3
8.20	39.0	57.0	8.01	43.8	60.9
10.05	47.0	56.0	10.01	45.7	60.8

temperature can be interpreted as an index to measure the progress of denaturation. In this regards, differential increase of  $k$  with respect to  $T$  (Fig. 3(c)) can be thought to represent the rate at which protein denaturation occurs when  $T$  is gradually increased.  $\Delta k$  is proportional to the quantity of unfolded protein accumulated up to that temperature. The interpretation of  $dk/dT$  (Fig. 3(c)) is thus similar to that of the endothermic heat-temperature curve in DSC analysis.

Denaturation of BSA occurred at  $T_i \leq T \leq T_s$  (Fig. 3). Accordingly, the denaturation temperature  $T_d$  can be defined in various ways. To be consistent with DSC data, in which  $T_d$  is generally taken as  $T$  at which endothermic heat flow is maximal,  $T_d$  was defined here as the temperature that corresponds to the maximal  $dk/dT$ , i.e., to the peaks in Fig. 3(c). Measured  $T_i$  and  $T_d$  data agree with DSC data within 5 °C except for  $T_d$  at pH = 5.05 (Table I). The relatively large discrepancy at pH = 5.05 is because denaturation occurs over a relatively large temperature range with relatively small change in thermal properties. Thus, the change in  $k$  can be used to probe the denaturation process. Because the change in  $k$  is believed to be mainly due to changes in BSA structure and to its dehydration, similar analysis may be possible for proteins other than BSA.

The reason that  $k_{sol}$  change during denaturation is that  $k_{BSA}$  changes as the protein unfolds. When the protein loses its tertiary structure, the vibrational energy transport mechanism of the protein also changes; as a consequence, its thermal properties also change. Changes in hydrogen bonding and non-polar hydrophobic interactions may be another reason for that the change in  $k_{BSA}$ . Folded proteins usually have a hydrophilic surface and a hydrophobic core. Charged or polar chains which occupy the surface of the protein interact with surrounding water. Intermolecular hydrogen bonds make the proteins stable in the solution. Minimizing the exposure of hydrophobic chains is one of the forces that drive protein folding. When a protein is denatured, it loses its three-dimensional structure and hydrogen bonds break; this results in loss of solubility and in communal aggregation. Communal aggregation reduces the total area exposed to water as hydrophobic amino acids bond to each other. We suggest that these changes cause the thermal network of a protein solution to change, which we believe would be common for some proteins other than BSA because most proteins

have common structural features. Nevertheless, further investigations are required to generalize the mechanism to other proteins.

In summary, we measured the change in  $k$  of aqueous solutions of BSA at different pH values while varying the temperature and the protein concentration. The  $k$  of BSA was experimentally determined based on the results. Variation of  $k$  with temperature showed a strong correlation between  $k$  and the denaturation state of the protein. These results suggest that thermophysical properties could be an effective tool to analyze the conformational dynamics of a protein, in the same way that thermodynamic properties are employed in the conventional DSC analysis. The method of protein analysis demonstrated in this work is also expected to be useful in micro-total analysis systems using proteins having similar characteristics because the measuring device can be easily miniaturized.

This work was supported by the Korean government (NRF-2011-0000109 and NRF-2011-0016489).

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