International Journal of Biological Macromolecules 52 (2013) 353–357

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Monitoring protein denaturation using thermal conductivity probe

Byoung Kyoo Park[a], Namwoo Yi[a], Jaesung Park[b], Dongsik Kim[a,*]

[a] Department of Mechanical Enigneering, POSTECH, Pohang 790-784, Republic of Korea
[b] School of Interdisciplinary Bioscience and Bioengineering, POSTECH, Pohang 790-784, Republic of Korea

ARTICLE INFO

Article history:
Received 31 May 2012
Received in revised form 25 August 2012
Accepted 10 October 2012
Available online 1 November 2012

Keywords:
Protein denaturation
Thermal analysis
Thermal conductivity
Three-omega method

ABSTRACT

We propose a method for probing denaturation of proteins by measuring the thermal conductivity of the solution. We use the three-omega method with a microfabricated ac thermal sensor to measure the thermal conductivity of lysozyme, β-lactoglobulin, and bovine serum albumin protein solutions over a range of temperature and pH. Results suggest that conformation transformation of the protein during denaturation changes the thermal network in protein solutions and thus changes the thermal conductivity for all the tested proteins. The proposed method of denaturation monitoring requires much simpler experimental setup than conventional methods such as differential scanning calorimetry and circular dichroism detection. We also demonstrate that the proposed analytical technique can detect the protein denaturation in real time. Consequently, it is expected to be useful in lab-on-a-chip (LoC) applications as the probe can be easily miniaturized for integration into LoC devices and allows real-time analysis.

1. Introduction

Proteins are essential components of biological cells. Proteins perform physiological activities including catalysis of chemical reactions, cell signaling, signaling transduction, immune response, and cell adhesion; some have structural functions [1,2]. Generally, proteins exist in a native state, which is the most stable three-dimensional folded structure, and their tertiary folded structure determines their specific functions [3]. Proteins that are exposed to external stress or to compounds may lose their tertiary structure, i.e., become denatured. Denaturation causes the protein to adopt a random coil conformation [4]. This structural change may disrupt cell activities, and can cause cell death. Understanding of the effects of external influences on denaturation may provide valuable physiological insight. A first step to such understanding is to detect denaturation accurately and in real time. To investigate the physics of conformational change in proteins, various methods have been employed to detect the denaturation state [5–21]. Differential scanning calorimetry (DSC) has been widely used to investigate the thermodynamics of protein denaturation [7–10,20,21]. DSC can measure the change in heat capacity and enthalpy of protein solutions during denaturation. However, insufficient resolution makes DSC inadequate for the dilute (0.1–10 mg ml^{-1}) protein solutions typical of biological systems. Also, the DSC curve changes with the scan rate of a device, so precisely determining denaturation temperature is difficult [20]. Far-ultraviolet (UV) circular dichroism (CD) is a powerful technique that is sensitive to the secondary structure of a protein. CD is based on differential absorption of left- and right-circularly polarized light; the signal reflects the protein's secondary structure. Far-UV CD is a powerful tool to investigate secondary structure of protein because specific structures such as the α-helix of protein and the double helix of DNA have their own representative CD spectral signatures [14–16]. By analyzing CD spectra, the fraction of conformations such as α-helix, β-sheet, β-turn, or random coil in the protein can be estimated. However, measurement of CD is complicated because oxygen and aqueous buffers often absorb light in the spectral range that exhibits the protein's structural features. Tris, dithiothreitol, histidine, and chloride buffers must be avoided, and phosphate, sulfate, carbonate, and acetate buffers can be used only when they are diluted to <50 mM. An alternative method of detecting protein denaturation that does not suffer from these limitations of DSC and CD would be a significant advance. Recently, there were several investigations concerning the feasibility of sensing biochemical reactions based on thermal properties' measurement [22–24].

Among the studies, we reported that thermal conductivity k [W m^{-1} K^{-1}] of bovine serum albumin (BSA) solutions has a strong correlation with conformational change of BSA [22]. k changes during denaturation for two reasons; change in the protein's structure causes changes in: (1) k of a protein itself because the vibrational energy transport mechanism is altered and (2) the thermal network in a solution including the thermal contact resistance between protein and the buffer. In its tertiary structure, a protein usually has a hydrophilic surface with a hydrophobic core. Intermolecular hydrogen bonds make a protein solution stable. However, protein denaturation changes the degree of hydrogen bonding and

* Corresponding author. Tel.: +82 54 279 2179; fax: +82 54 279 3199.
E-mail address: dskim87@postech.ac.kr (D. Kim).

0141-8130/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2012.10.027

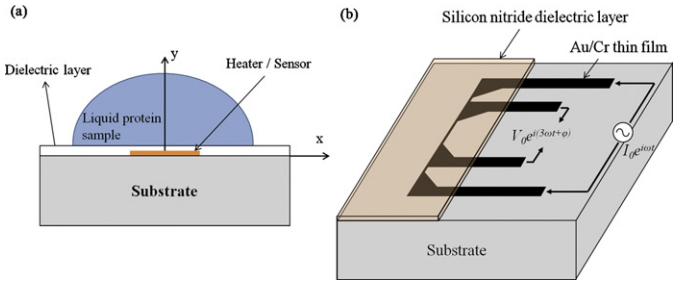

Fig. 1. (a) Schematic diagram of 3ω technique for measuring k of liquid sample and (b) schematic diagram of thermal conductivity sensor.

non-polar hydrophobic interaction; these changes reduce the protein's solubility and communal aggregation. We suggested that these mechanisms make k of a protein solution (k_{sol}) change. Nevertheless, that study [22] was restricted to only a single protein, BSA, so further investigations are required to generalize the conclusion that the conformational dynamics of common proteins can be monitored by measuring k_{sol}.

In the present work, k_{sol} was measured in solutions of β-lactoglobulin and lysozyme which are structurally distinct from BSA. Whereas the structure of BSA is dominated by α-helix, β-lactoglobulin has 10–15% α-helix and 43% β-sheet, and lysozyme has 40% α-helix, 12% β-sheet, and 48% random coil [25]. The present work also introduces a measurement scheme that can be used to monitor protein denaturation in real time.

2. Materials and methods

2.1. Sample preparation

β-Lactoglobulin (Sigma–Aldrich L3908) was obtained as a chromatographically purified lyophilized powder from bovine milk. It was dissolved (2.72×10^{-3} M, 50 mg ml^{-1}) in distilled water; the pH of the solution was adjusted to 7.00, then stirred for 2 h. Lysozyme solution was prepared by the protein obtained from chicken egg white (Sigma–Aldrich, 62971). It consists of a single 14.7-kDa polypeptide chain. The solution was dissolved (3.17×10^{-4} M, 2.18 mg ml^{-1}) in 50-mM sodium phosphate buffer with pH 6.20. And the solution was prepared daily. The two concentrations were chosen to compare the results with the existing DSC or far-UV CD data [16,19].

2.2. Measurement

To measure k_{sol} and k of base fluid k_b we used the three-omega method [26–31], which is a well-known technique to measure heat diffusion in bulk solid, solid thin films or liquid, and is particularly suited for measuring k of liquid samples which are electrically conducting or as small as sub-nanoliter in volume [29–31]. The sensing device is composed of a thin-film metal strip and a dielectric layer on a glass substrate (Fig. 1). The metal strip serves as a line heater as well as a temperature sensor. When ac current of frequency ω [s^{-1}] is applied to the line heater, it generates Joule heating at the frequency 2ω. Thus, temperature T and resistance of the heater also oscillate at the same frequency 2ω. The resistance multiplied by original current produces the 3ω voltage signal. The average thermal response of the sensor, more precisely, the amplitude of temperature oscillation can be expressed analytically as a function of the thermal properties of the substrate and the liquid sample in contact with the sensor [29]. The response is reduced to a simple form as a function of the qb, i.e., the half-width of the heater in units of q^{-1} under the condition that specific heat C and k are real-valued quantities [26]:

$$\Delta T \frac{\pi l k}{P} = -\ln(qb) + \eta(qb) - \frac{\pi}{4} i, \tag{1}$$

where P [W] is heating power, l [m] is the length of the heater, b [m] is the half width of the heater, and $q = \sqrt{2\omega/\alpha}$ is the inverse of thermal penetration depth where α [m^2 s^{-1}] is thermal diffusivity. Because $\eta(qb)$ approaches a real constant for $qb \ll 1$, k of a liquid sample k_l can be determined in two ways, the slope method and the single-frequency method.

2.2.1. Slope method

To obtain k of a liquid sample k_l using the slope method that is conventionally used, temperature responses of the sensor should be obtained by varying the modulation frequency. The thermal conductivity of the liquid sample k_l can be determined from the slope of the thermal-response vs. modulation frequency curve in a plot of the real (in-phase) part of temperature T_{real} vs. $\ln(\omega)$ [29]; neglecting boundary mismatch, the measured k can be approximated as $k_s + k_l$:

$$k = k_s + k_l = -\frac{P}{2l\pi} \frac{\mathrm{d}\ \ln\omega}{\mathrm{d}T_{real}} \tag{2}$$

where k_s is the k of the sensor substrate (1.083 W m^{-1} K^{-1} for Borofloat glass substrate) [32]. Although substrates with lower thermal conductivities give better sensitivity, glass was chosen to be the substrate material for easy fabrication.

2.2.2. Single-frequency method

Alternatively, k_l can be determined by using the imaginary (out-of-phase) part of temperature oscillation T_{imag} at a single frequency; in this method, measured k can be also replaced by $k_s + k_l$ [31]:

$$k = k_s + k_l = -\frac{P}{4l} \frac{1}{\mathrm{d}T_{imag}}, \tag{3}$$

The slope method is generally more sensitive than the single-frequency method because the real part of the temperature response has much greater magnitude than does the imaginary part. Nevertheless, it is advantageous in small volume measurement like sub-nanoliter scale liquid [31]. Also, we can get k in real-time because this scheme yields k from the temperature response of single frequency without scanning temperature responses of a certain range of frequencies.

2.3. Experimental methods

The lift-off process was used to fabricate a thin-film Au/Cr strip heater/sensor on a Borofloat glass substrate (Schott 33). A 200 nm-thick Au film was deposited with a 20-nm-thick chromium layer as an adhesion layer using an electron beam evaporator. The width and length of the metal strip are 20 μm and 1 mm, respectively. After patterning the heater/sensor, the PECVD (plasma-enhanced chemical vapor deposition) process was employed to deposit a silicon nitride (Si_3N_4) dielectric layer of 200 nm in thickness for electrical insulation of the heater/sensor. An electrical current of frequency ω from an internal source of a lock-in amplifier (Stanford Research Systems, model SR810) was supplied to the Au strip. The ω component of the signal was eliminated by adjusting the gain of a differential amplifier (Analog Devices, model AD620) with an input impedance 10 GΩ, leaving only the 3ω signal that bears the thermal information. The 3ω signal was measured using the lock-in amplifier, and the amplitude and phase of the temperature signal were determined from the signal. The measurement was repeated by varying the modulation frequency from 5000 to 1.5 Hz. In the case of DI water, the thermal penetration depth at