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RESEARCH ARTICLE

Determining the degree of denaturation of bovine serum albumin using a new UV analysis technique

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Abstract: There is a lack of fast and inexpensive analysis methods to study the conformational changes and the degree of denaturation of proteins quantitatively. As such, a novel analytical technique is developed based on the ultraviolet-visible (UV-Vis) absorption spectrum of proteins, and a mathematical modeling of the results. The phenomenon behind this technique is the shift of the absorption peak of amino acid residues of BSA such as tyrosine, phenylalanine, and tryptophan as the protein unfolds and these residues are exposed to the solvent. However, the portion of the peak that is shifted is miniscule and it can be enhanced by using the proposed technique in this paper. As an example, we also show how this technique was applied for evaluating the temperature effects on thermal denaturation of bovine serum albumin (BSA) protein. A degree of denaturation curve as a function of time was obtained at three different temperatures using this technique. The results are reproducible and consistent with those reported in the literature. This technique is especially recommended for analyses where several tests are needed quickly, and the amount of sample is limited. Among the applications, it can be used for evaluation of disinfection through assessing the degree of denaturation for pathogens proteins.

Keywords: UV-vis spectroscopy, protein denaturation, bovine serum albumin, tyrosine, phenylalanine, tryptophan

1 Introduction

Proteins are extremely diverse in function, with their respective biological properties depending on physical interactions with other molecules or ligands. These interactions are governed by hydrogen bonding, van der Waal's forces, electro-static forces, and other non-covalent interactions [1]. As such, all proteins can bind to other molecules, albeit selectively under different conditions with a range of binding affinities [1]. The contours of a protein which are capable of interactions are often referred to as binding sites. Here, the conformation of the protein determined by amino acid sequences often governs the protein shape and selectivity toward ligands [1]. For example, in the case of pathogens, the SARS-CoV (2002 strain) vs SARS-CoV-2, it's seen that the latter has a binding affinity towards the human ACE2 receptor that is greater than SARS-CoV up to a factor of 20 [2, 4]. Variations in the amino acid sequence between these two protein structures result in this binding difference [3, 4]. With this said, it may be also be of interest to those studying protein-ligand interactions to determine protein denaturation/stability under varying environmental conditions. This could be valuable in treatment development for disrupting virus-human protein interactions. Such changes could occur with variations in temperature, pH, and electric field. Several techniques can be used to observe these changes, such as circular dichroism (CD), X-ray crystallography, nuclear magnetic resonance (NMR), Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) [5, 6]. Some techniques in particular, X-ray crystallography, NMR, and DSC, are relatively time consuming, complex, and require expensive instruments. UV absorption analysis is a very fast, cheap, quantitative, and an accurate optical spectroscopy technique that requires only a small amount of sample. This is of more importance when frequent sample analyses are needed or when the amount of sample is limited. However, the conformational changes in proteins manifest very small changes in the UV spectra that are difficult to discern by the traditional UV analysis techniques.

Proteins in general, have amino acid residues such as tyrosine (Tyr), phenylalanine (Phe) and tryptophan (Trp). The aromatic chains of these residues have strong UV absorption in the range of 240-300 nm [7, 8]. Here, we select bovine serum albumin (BSA) as a model

protein to measure the effect of time, under an isothermal temperature condition, on its degree of denaturation. With UV-vis spectroscopy, a characteristic peak can be seen at ∼278 nm, likely due to the presence of such amino acid residues [9]. During the denaturation of a protein, as the protein unfolds, some of these residues are exposed to the solvent and are perturbed. This will cause absorption of higher energy photons and shifting of the absorption spectrum to the left [10]. However, in an attempt to measure denaturation over short periods of time (up to 60 min) with UV-vis, the portion of the UV spectrum that shifts to the left is slight and difficult to observe. Furthermore, studying changes in denaturation by UV-vis can mostly be described qualitatively, noting that an effect may or may not be happening, but to what degree is unknown unless more tasking techniques are implemented, as described above. In this article, we introduce a novel UV analysis technique and mathematical approach to amplify these small changes in the spectra and assess the degree of denaturation of proteins quantitatively.

2 Materials and methods

BSA protein sample was purchased from Thermo Fisher Scientific and was diluted with deionized water to yield a sample solution at about 2.5×10^{-5} M concentration. The UV spectra measurements were performed using UV-Vis spectrophotometer Evolution 600 by Thermo Scientific. All the data analysis on the raw spectra were done and all the graphs were made using Python.

First, the UV spectrum of the sample solution was obtained at room temperature. The sample was then transferred to a sealed container and placed in a water bath at 65°C for a specific amount of time. Immediately after the thermal bath, the sample was quenched in cold water for 1 min and left at room temperature for 2 min to reach the thermal equilibrium. The UV spectrum was then obtained within a minute and sample was transferred to the thermal bath again. The spectra were taken repeatedly for five cumulative incubation times: 5, 10, 20, 40 and 60 minutes.

3 Results and discussion

As shown in Figure 1, the UV spectra of BSA in its natural form and after the thermal incubations exhibit very small changes in the shape of the curves. The main absorption peak of the protein residues can be seen centered at around 278 nm.

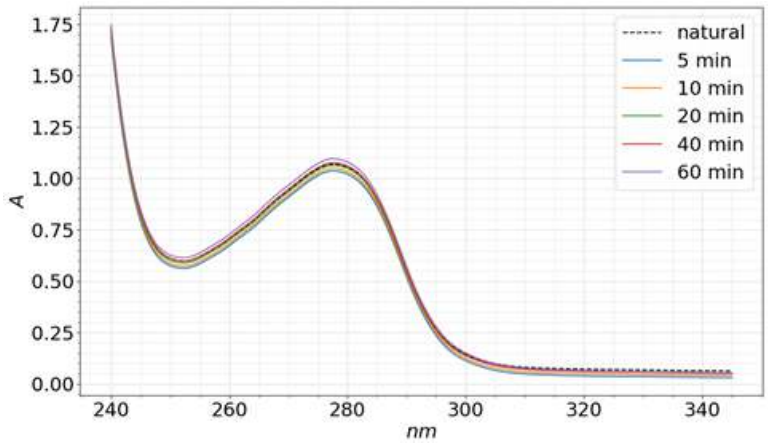

Figure 1 The UV absorption spectra of BSA in natural state and after thermal incubation at 65°C for different times.

To magnify the small changes and find the center of the small denaturation shifts, a mathematical analysis was performed to calculate the ratio of each thermal curve to the natural form. The analyzed data show that the shifted spectrum is centered at around 252 nm as shown in Figure 2.

As such, the portion of main broad peak that has shifted to the left was measured. To that end, the analysis was done based on the ratios of the shifted peaks (at 252 nm) to the main peaks (at 278 nm) for each incubation time. First, the ratio of the absorption at 278 nm versus that of

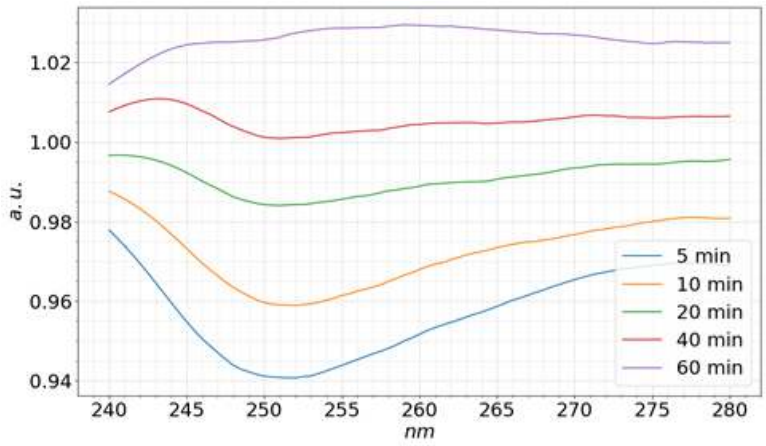

Figure 2 Magnifying the small changes in the spectrum shifts of BSA after thermal incubation for different times (arbitrary unit y-axis).

252 was calculated for each incubation time:

$$r_i = \frac{A_i\,(278)}{A_i\,(252)}$$

where r_i is the calculated ratio for incubation time i, and $A\,(\lambda)$ is the absorption at wavelength λ. Then, each ratio was compared with the ratio for the natural state of the protein as shown in the pseudocode below:

$$For\ i = 1\ ..\ n: \quad D_i = r_i - r_0$$

where D_i is the degree of denaturation for the incubation time i, and r_0 is the ratio for the natural state.

The result of this data analysis showing the degree of denaturation after normalization is demonstrated in Figure 3. The curve forms an exponential function and after 60 min incubation, the degree of denaturation is predicted to reach close to its final equilibrium state. The fitted curve falls within a bounded exponential function as shown below:

$$f\,(t) = 1 - e^{-\frac{t}{20.8}}$$

where $t$is time in minutes, $f\,(t)$is a function of time showing the degree of denaturation, and 20.8 is the time constant. Time constant is when the degree of denaturation reaches about 0.63 (or $1 - \frac{1}{e}$).

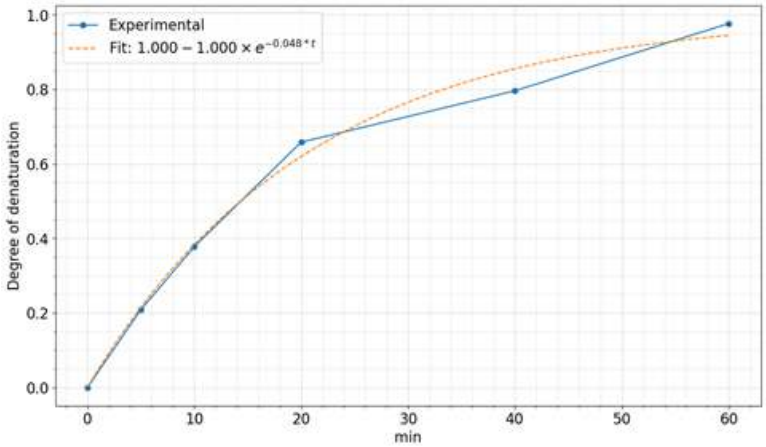

Figure 3 The degree of denaturation of BSA protein after thermal incubation at 65°C for different incubation times after normalization. The dashed curve shows the exponential fit of denaturation over time.

4 Conclusion

All the data analysis described here can be automated using a programming language to generate the final output fast and conveniently. The new analytical technique promises a fast and easy method of measuring the conformational changes in proteins using a simple UV-vis spectrometer.

This technique can be applied on various types of proteins for disinfection assessment purposes. Proteins of pathogens can be tested using this technique on surfaces or in solutions when they undergo any environmental stress including thermal, electromagnetic, and chemical. For example in the case of SARS-CoV-2, quantification of the degree of denaturation for the spike protein will give us a measure of viable virus titer. If the spike protein is denatured, the virus cannot bind to the human ACE2 receptor, therefore preventing infection of human cells [11].

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Conflict of interest

The authors declare no conflict of interest.

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