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Secondary structure investigation of bovine serum albumin (BSA) by Fourier transform infrared (FTIR) spectroscopy in the amide III region

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ABSTRACT

Fourier transform infrared spectroscopy is widely used to analyze protein secondary structures. The common strategy in this field is to analyze the conformation sensitive 1700-1600 cm^{-1} amide I region of protein FTIR spectrum. Though the amide III region of protein is also sensitive to secondary structural changes, its potential for protein secondary structural analysis is largely unexplored. In this paper, we performed a detailed investigation on the second structural analysis of bovine serum albumin by monitoring the spectral variation of the amide III band under a variety of pH conditions by FTIR spectroscopy and FTIR second derivative spectroscopy. Our results show that both acidic and basic conditions have pronounced effects on the overall secondary structures of BSA, suggesting denaturation effects. Furthermore, we observe that the amide III band profiles under acidic and basic conditions appear to be quite different. Our results clearly demonstrate that the amide III region is a promising probing region for protein secondary structural analysis.

1. Introduction

Fourier transform infrared spectroscopy is widely used to analyze protein secondary structures [1-6]. The 1700-1600 cm^{-1} amide I region is commonly used in the endeavor. Different protein secondary structures have different absorption frequencies in this region. For example, the 1657-1648 cm^{-1} region is believed to correspond to α-helix structure; the 1641-1623 cm^{-1} region is believed to correspond to β-sheet structure; the 1657-1642 cm^{-1} region is believed to correspond to unordered structure; the 1686-1662 cm^{-1} region is believed to correspond to turn structure [5]. The amide I band thus can be decomposed into different component bands corresponding to different secondary structures by curve-fitting method. Fraction calculations of fitted component peaks offer a quantitative picture about protein secondary structures. Though the amide I region is often used in protein secondary structural analysis, it has two technical limitations. First, the atmospheric water vapor absorptions in the amide I region can significantly interfere with the amide I absorption and the removal of water vapor is a challenging task. Secondly, when studying protein in aqueous solution, the interference from the strong absorption of water bending mode at 1640 cm^{-1} requires the use of very thin infrared sample cell, this adds extra

technical challenge. Yet, the above two limitations can be avoided if we use amide III region to probe protein secondary structures.

The amide III region is located in the 1400-1200 cm^{-1} region and it is also sensitive to protein secondary structural changes [5,7-10]. Previously, Anderle *et al.* studies heat denaturation effect on protein secondary structure using FTIR spectroscopy in amide III region [7]. Cai *et al.* explored to use chemometric analysis in the amide III region for protein secondary structural analysis [10]. Two reasons make amide III less employed in protein secondary structural analysis. First, the spectral assignment in this region is not that well developed if compared with that in the amide I region. Second, the extinction coefficient of amide III mode is usually small, thus requires a concentrated protein solution. Despite the limitations, the amide III band has two unique advantages over the amide I region when used for protein secondary structural analysis. First, this region is basically free from water vapor interference. Second, this region is free from the absorption of solvent water. In addition, the amide III region is a useful probe region for protein conformational change in Raman spectroscopy [11]. Therefore, it is still very attractive to use amide III region for protein secondary structural analysis. In this study, we aim to explore the potential of the amide III

region for the secondary structural analysis. We chose bovine serum albumin (BSA) as the model protein for this showcase purpose as BSA is commonly used model protein in the field of protein science due to its well characterized structure and its low cost. The secondary structures of bovine serum albumin (BSA) under different pH conditions were investigated by FTIR spectroscopy and FTIR second derivative spectroscopy in the amide III region. Furthermore, we would like to compare how acidic condition and basic condition affect the secondary structures of BSA differently.

2. Experimental

2.1. Materials

Bovine serum albumin (BSA) with a purity of 98% (Catalog number: 735094) was obtained from Roche (Switzerland). Deionized water is from a Millipore system (Billerica, USA). Sodium hydroxide (NaOH) and hydrochloride acid (HCl) in analytical grade were from local vendors. BSA solution was prepared by dissolving lyophilized BSA powder in H_2O. The pH of BSA aqueous solution was adjusted by adding concentrated HCl or NaOH solutions. The exact pH of BSA solution was determined with a pH meter. The concentration of BSA solution was determined by weighing to be 50 mg/mL.

2.2. Instrumentation

The BSA FTIR spectra were obtained with a Bruker Vertex 70 FTIR spectrometer (Bruker, Germany) equipped with a DTGS detector. All measurements were performed under ambient conditions. A demountable liquid cell (PIKE Technologies, USA) with a pair of circular CaF_2 windows and a 25 μm spacer was used. Spectra were collected with a spectral resolution of 4 cm^{-1} and 128 scans. FTIR data processing such as solvent subtraction and second derivative treatment was performed with OPUS software (Version 7.2, Bruker, Germany).

3. Results and discussion

The amide III mode of a protein is the in-phase combination of the N-H bending and the C-N stretching vibration with small contributions from the C=O in plane bending and the C-C stretching vibration [5]. Like amide I mode, different protein secondary structures have different amide III absorptions. Based on the general assignments made by Griebenow and Klibanov, an amide III absorption between 1320 and 1300 cm^{-1} corresponds to α-helix structure; an amide III absorption between 1280 and 1260 cm^{-1} corresponds to unordered structure; an amide III absorption between 1250 and 1240 cm^{-1} corresponds to extended chain; an amide III between 1240 and 1220 cm^{-1} corresponds to β-sheet structure [8].

Figure 1 shows the amide III band variations under difference acidic pH values for BSA. The pH = 6.3 spectrum is the spectrum of BSA in neat water and is considered to the spectrum of BSA under native condition. Native BSA is an α-helix protein with 50% of its structure being α-helix [7]. As expected, the amide III spectrum of BSA is dominated by the ~1310 cm^{-1} band with other bands below 1300 cm^{-1} corresponding to unordered and extended chain structures. With the decrease of pH value from 6.3 to 0.8, the absorbance around 1310 cm^{-1} decreases and the absorbance below 1300 cm^{-1} increases correspondingly. This suggests that acidic pH destruct native α-helix structure of BSA. Our observation is consistent with previous study about acid denaturation of BSA by FTIR spectroscopy in amide I region [12]. In other words, acidic pH has a denaturing effect on BSA. The above analysis is certainly a very qualitative analysis. To gain more insights into the denaturation of BSA under acidic conditions, we further

performed second derivative analyses of the amide III spectra, as shown in Figure 2, Figure 3, and Table 1.

Second derivative technique is a resolution-enhancement technique commonly used to resolve the overlapped peaks in the amide I and amide III bands for protein secondary structure analysis [3,6,8,13]. All the spectra in Figure 1 were subjected to second derivative treatment. Yet, to give a better illustration, only several representative second derivative spectra were shown in Figure 2. In addition, the second derivative results for all of the spectra in Figure 1 were listed in Table 1 which includes the resolved peak frequencies and their spectral assignments. Figure 3 shows how the second derivative peak intensities of different BSA secondary structures change with pH values. Clearly, Figure 2, Figure 3, and Table 1 give us a more quantitative view about how acidic condition affects the secondary structures of BSA. As we can see, with the decrease of pH from 6.3 to 0.8, both the 1318 cm^{-1} and 1301 cm^{-1} peak corresponds to α-helix structures decrease their intensities in second derivative spectra, suggesting the destruction of α-helix structure. The 1243 cm^{-1} peak that corresponds to extended chain structure also decreases its intensity in second derivative spectra, suggesting the destruction of the extended chain structure. The 1268 cm^{-1} peak, which corresponds to unorder structure increases its intensity in second derivative spectra, suggesting the denaturation of BSA. Furthermore, as indicated in Table 1, the 1318 cm^{-1} peak shifts to 1316 cm^{-1} position and the 1268 cm^{-1} peak shifts to 1266 cm^{-1} position at pH = 0.8. By contrast, the 1301 cm^{-1} peak and 1243 cm^{-1} peak remain unchanged with respect to frequency. Overall, the acidic condition denatures BSA partially since part of the α-helix structure still remains at the extreme pH value of 0.8.
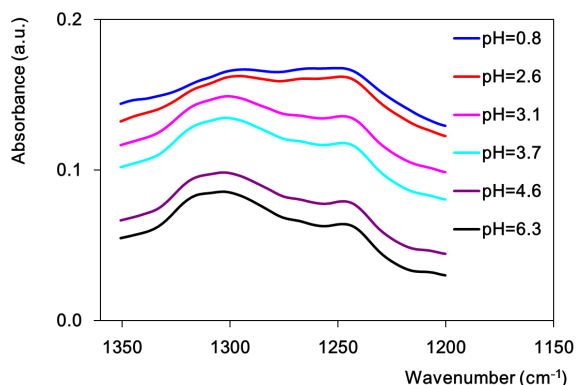

Figure 1. FTIR absorption spectra of BSA in the amide III region under native (pH = 6.3) and different acidic conditions.
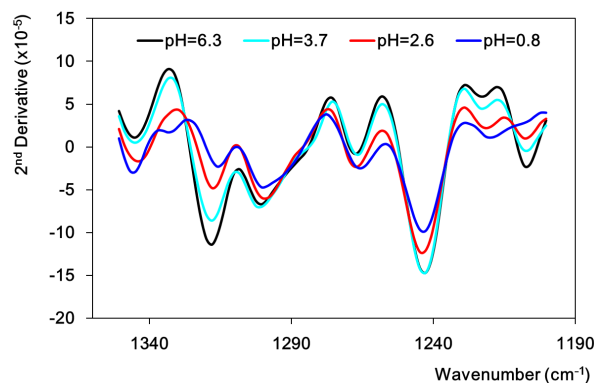

Figure 2. Second derivative spectra of BSA in the amide III region under native (pH = 6.3) and different acidic conditions.

Table 1. Frequencies and assignments of the resolved peaks in second derivative spectra under native (pH = 6.3) and different acidic conditions.

pH	α-Helix (cm⁻¹)	α-Helix (cm⁻¹)	Unordered (cm⁻¹)	Extended chain (cm⁻¹)
6.3	1318	1301	1268	1243
4.6	1318	1301	1268	1243
3.7	1318	1301	1268	1243
3.1	1318	1301	1268	1243
2.6	1318	1301	1268	1243
0.8	1316	1301	1266	1243

Table 2. Frequencies and assignments of the resolved peaks in second derivative spectra under native (pH = 6.3) and different basic conditions.

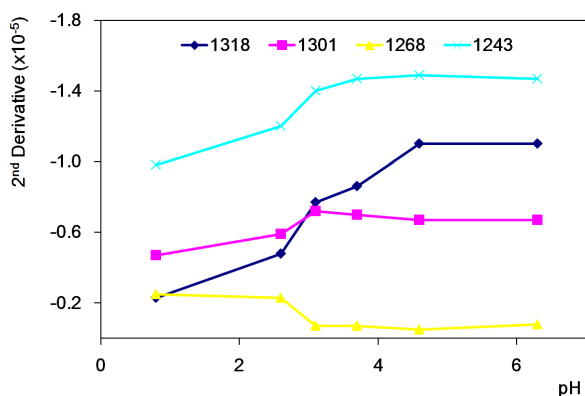| pH	α-Helix (cm⁻¹)	α-Helix (cm⁻¹)	Unordered (cm⁻¹)	Extended chain (cm⁻¹)
6.3	1318	1301	1268	1243
8.2	1318	1300	1268	1243
10.4	1318	1300	1268	1243
11.6	1318	1300	1268	1243
12.5	1318	-	1270	1243
13.5	1318	-	1270	1243

Figure 3. Second derivative intensity variations of different secondary structures under native (pH = 6.3) and different acidic conditions.

Figure 4 shows the amide III bands under difference basic pH values for BSA. The pH = 6.3 spectrum of the native BSA is re-shown here for comparison. Under mild basic conditions below pH = 11.6, the spectral shape did not change significantly compared with that of native BSA spectrum at pH = 6.3.
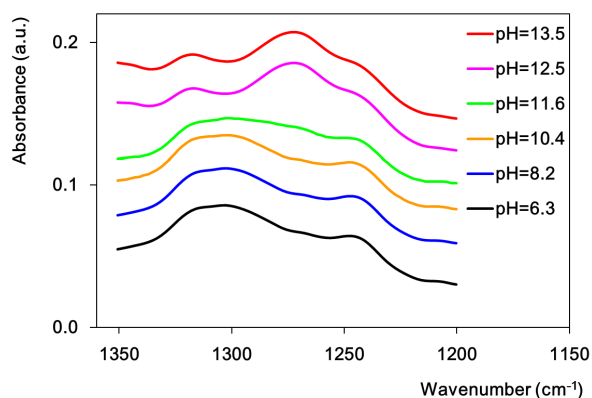
Figure 4. FTIR absorption spectra of BSA in the amide III region under native (pH = 6.3) and different acidic basic conditions.

Above pH = 11.6, the absorbance in the spectral region above 1300 cm⁻¹ decreases significantly with the increase of pH value and the absorbance below 1300 cm⁻¹ increases significantly. This suggests that basic pH destruct native α-helix structure of BSA and has a denaturing effect on BSA. To obtain a better understanding of the denaturation of BSA under basic conditions, we further performed more quantitative analysis

with second derivative technique on the amide III spectra shown in Figure 4.

All the spectra in Figure 4 were subjected to second derivative treatments. The frequencies and peak assignments from the second derivative results were listed in Table 2. Figure 5 shows the second derivative spectra of BSA under several representative basic pH conditions. Figure 6 shows how the second derivative peak intensities of different BSA secondary structures change with basic pH values. Figure 5, Figure 6, and Table 2 give us a more quantitative view about how basic pH affects the secondary structure of BSA. As we can see, with the increase of pH from 6.3 to 13.5, the 1301 cm⁻¹ peak, which corresponds to one of the two α-helix structures decrease its intensities in second derivative and eventually disappears when pH reaches 12.5. This suggests a more significant destruction of the native α-helix structure. The 1243 cm⁻¹ peak which corresponds to extended chain structure also decreases its intensity in second derivative spectra, suggesting the destruction of the extended chain structure. The 1268 cm⁻¹ peak, which corresponds to unordered structure increases its intensity in second derivative spectra, consistent with the overall picture of BSA denaturation. The 1318 cm⁻¹ peak intensity in second derivative remains basically unchanged. Furthermore, as shown in Table 2, the 1268 cm⁻¹ peak shifts to 1270 cm⁻¹ position and the 1301 cm⁻¹ peak shifts to 1300 cm⁻¹ position at pH = 13.5. By contrast, the 1318 cm⁻¹ peak and 1243 cm⁻¹ peak remain unchanged. The presence of 1318 cm⁻¹ peak even at extreme basic pH of 13.5 suggests that BSA is not completely denatured even under such strong basic environment.
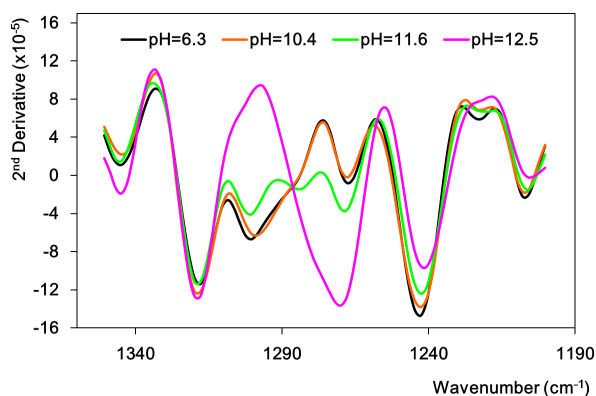
Figure 5. Second derivative spectra of BSA in the amide III region under native (pH = 6.3) and different basic conditions.

In Figure 7, we performed a comparison of BSA under native, acidic, and basic condition. The three spectra have been

shown in Figure 2 and Figure 5, but reshown here for better illustration. The three spectra correspond to BSA under three difference states: native state, acid-denatured state, and base-denatured state. From the comparison, it is clearly that basic condition denatured BSA more significantly as evidenced by the disappearance of the 1301 cm^{-1} α-helix peak under the extreme basic pH of 12.5. By contrast, the denaturation effect of acid on BSA is relatively mild.
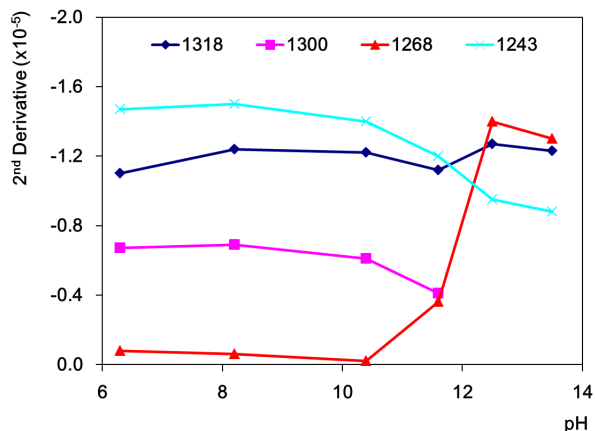

Figure 6. Second derivative intensity variations of different secondary structures under native (pH = 6.3) and different basic conditions.
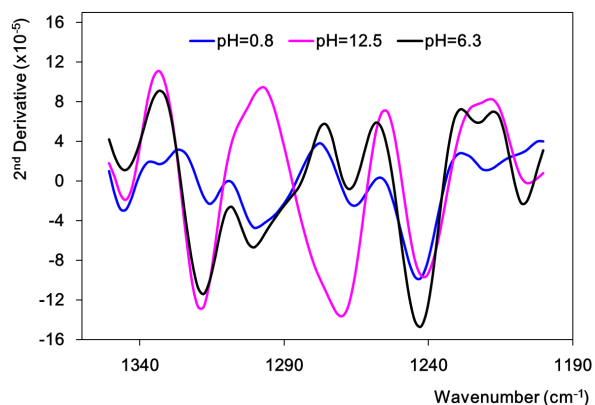

Figure 7. Second derivative spectral comparison of BSA under native (pH = 6.3), acidic (pH = 0.8), and basic (pH = 12.5) conditions.

4. Conclusion

An investigation on the second structural analysis of BSA under different acidic and basic conditions was performed using FTIR spectroscopy and FTIR second derivative spectroscopy in the amide III region. We show that both acidic and basic conditions have denaturation effects on BSA native structures, though to different extents. Our results clearly demonstrate that the amide III region is a sensitive probing region for protein secondary structural analysis.

References

[1]. Byler, D. M.; Susi, H. *Biopolymers* **1986**, *25*, 469-487.
[2]. Surewicz, W. K.; Mantsch, H. H. *Biochim. Biophys. Acta* **1988**, *952*, 115-130.
[3]. Dong, A.; Huang, P.; Caughey, W. S. *Biochemistry* **1990**, *29*, 3303-3308.
[4]. Goormaghtigh, E.; Cabiaux, V.; Ruysschaert, J. M. *Subcell. Biochem.* **1994**, *23*, 405-450.
[5]. Barth, A.; Zscherp, C. Q. *Rev. Biophys.* **2002**, *35*, 369-430.
[6]. Kong, J.; Yu, S. *Acta Biochim. Biophys. Sin.* **2007**, *39*, 549-559.
[7]. Anderle, G.; Mendelsohn, R. *Biophys. J.* **1987**, *52*, 69-74.
[8]. Griebenow, K.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U. S. A.* **1995**, *92*, 10969-10976.
[9]. Vedantham, G.; Sparks, H. G.; Sane, S. U.; Tzannis, S.; Przybycien, T. M. *Anal. Biochem.* **2000**, *285*, 33-49.
[10]. Cai, S.; Singh, B. R. *Biochemistry* **2004**, *43*, 2541-2549.
[11]. Li-Chan, E. C. *Le Lait* **2007**, *87*, 443-458.
[12]. Estey, T.; Kang, J.; Schwendeman, S. P.; Carpenter, J. F. *J. Pharm. Sci.* **2006**, *95*, 1626-1639.
[13]. Susi, H.; Byler, M. D. *Biochem. Biophys. Res. Commun.* **1983**, *115*, 391-397.