The effect of Arginine, Lysine and Histidine in the myosin secondary structure by circular dichroism and Raman spectroscopy

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ABSTRACT
Amino acids containing L-Arginine (Arg), L-Lysine (Lys) and L-Histidine (His) are recognized to increase the solubility of myosin at low ionic strength, but there is a difference in the researchers’ views on their effects on the secondary structure of myosin. To elucidate which method is relatively appropriate for the determination of secondary structures of myosin added with Arg, Lys or His, Circular dichroism (CD) spectra and Raman spectroscopy were carried out respectively. Raman spectroscopy was more suitable than CD method for determination of the secondary structures of myosin added with His, while CD spectra was more accurate for the myosin samples added with Lys and the adequate concentration of Lys should be under 10 mM. Both CD and Raman methods could be applied to myosin added with Arg, however, the latter was recommended and the adequate additive content of Arg in CD method should be less than 10 mM.

Efecto de la arginina, la lisina y la histidina en la estructura secundaria de la miosina medido mediante dicroísmo circular y espectroscopia Raman

RESUMEN
Si bien se reconoce que los aminoácidos que contienen L-arginina (Arg), L-lisina (Lys) y L-histidina (His) aumentan la solubilidad de la miosina a baja fuerza iónica, en las opiniones de los investigadores existen diferencias respecto a sus efectos en la estructura secundaria de la miosina. Para dilucidar qué método es relativamente apropiado para determinar las estructuras secundarias de la miosina añadida con Arg, Lys o His, se aplicó el método de espectros de dicroísmo circular (CD) y la espectroscopia Raman. Esta última fue más adecuada que el método de CD para determinar las estructuras secundarias de la miosina agregada con His, mientras que los espectros de CD fueron más precisos para determinar estas estructuras en las muestras de miosina agregadas con Lys; además, se constató que la concentración adecuada de Lys debe ser inferior a 10 mM. Los métodos de CD y espectroscopia Raman podrían aplicarse a la miosina agregada con Arg; sin embargo, después de comprobar que el contenido de aditivo adecuado de Arg en el método de CD debe ser inferior a 10 mM, se recomendó el último.

1. Introduction
The myosin, with a ratio of 55%-60% in myofibrillar proteins, is a major component of fish muscle (Lanier, Carvajal, Yongsawatdigul, & Park, 2005). The solubility of myosin is closely related to its physicochemical properties, such as gelation, emulsification and so on. However, myosin is soluble only under high salt conditions (>0.3 M). Protein secondary structure is related to the atom arrangement in the main chain of the polypeptide, which is independent of the side chain conformation. Secondary structural components consist of α-helix, β-sheet, β-corner and random coil, etc., which compose the essential factors of the high protein structure (Zhang, Zhang, & Wang, 2015).

Many methods such as nuclear magnetic resonance spectroscopy (NMR) (Wishart, Sykes, & Richards, 1992), circular dichroism (CD) spectra (Liu, Zhao, Xiong, Xie, & Qin, 2008), X-ray (Gao, Qiang, & Yang, 2009), fourier transform infrared spectroscopy (FTIR) (Kumosinski & Unruh, 1996) and Raman spectroscopy (Kirschner, Ofstad, Skarpeid, Høst, & Kohler, 2004) are usually used to detect the protein secondary structures. Among which, CD and Raman spectra are the two most common measures. CD spectroscopy is used for the determination of protein solutions with lower concentration (Liu et al., 2008). Raman spectroscopy is suitable for dry solid powders and it is non-destructive (Zhang et al., 2015).

Arg, Lys and His applied to increase the solubility of myosin at low ionic strength have received more attention (Chen et al., 2016; Gao, Wang, Mou, Shi, & Yuan, 2018; Guo, Peng, Zhang, Liu, & Cui, 2015; Takai, Yoshizawa, Ejima, Arakawa, & Shiraki, 2013). However, the mechanism of Arg, Lys and His for improving the solubility of myosin in low ionic strength solution remains unclear. Some works indicated that the solubility of myosin was enhanced by 5 mM Lys and His through changing the secondary structures of myosin (Chen et al., 2016; Guo et al., 2015). However, this appears to contradict Takai’s (2013) theory that myosin in the presence of 50 mM Arg or Lys with 50–300 mM NaCl showed an identical structure with which in the presence of...
350 mM NaCl. Essentially, both of these two distinct conclusions were obtained from CD spectra. Furthermore, in our previous work, we found the determination of myosin secondary structures using CD was disturbed by some amino acids. Therefore, to determine the secondary structure of myosin, it may be necessary to select different detection methods depending on the type of amino acid added and the amount of addition. In order to accurately determine the change of secondary structures of myosin induced by different amino acids, we compared the methods of CD and Raman.

2. Materials and methods

2.1. Reagents and materials

Live bighead carp was obtained from Auchan supermarket at Xuefu Road, Zhenjiang, China. L-Arginine (Arg) and L-Lysine (Lys) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). L-Histidine (His) was purchased from Aladdin Industrial Corporation (Shanghai, China). All chemicals used were of reagent grade.

2.2. Extraction of myosin

Myosin was extracted from the dorsal muscle of the bighead carp as previously reported (Yuan, Liu, Ge, Feng, & Gao, 2017). Analysis using sodium-dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) showed that the purity of extracted myosin was over 90% myosin. The myosin solution was stored at 4°C and used within 3 days.

2.3. Preparation of samples

Myosin solutions were diluted to 0.1 mg/mL for the measurements of CD spectroscopy and the lyophilized powder of 0.1 g was prepared for Raman spectroscopy, respectively. Furthermore, amino acids (Arg, Lys or His) with different final concentrations (0, 1, 5, 10 and 20 mM) dissolved at the same buffer solution were measured as well using the same method as myosin samples. Myosin solutions containing various concentrations of different amino acids were also prepared for the measurements of CD spectra.

2.4. CD spectroscopy

The CD spectrum was measured using a Jasco J-815 Spectrometer (Jasco Co. Ltd., Tokyo, Japan) with a 1 mm path length quartz cell. The scan range was 200–250 nm. Firstly, the CD spectra of myosin, Arg, Lys and His were obtained respectively. Secondly, we scanned the CD spectra of the mixtures of myosin with amino acids, called myosin-Arg, myosin-Lys and myosin-His, respectively. Furthermore, all of the CD spectra were corrected by subtracting the spectra of the buffer solution.

2.5. Raman spectroscopy

The Raman spectrum was detected by a DXR Laser Raman Spectrometer (ThermoFisher Co. Ltd., Waltham, MA, USA) with the sample placed on a smooth glass sheet. The scan range was 400–3000 cm⁻¹, the laser energy was 10 mW, the number of the spectra selected was 30 times and the resolution was 2 cm⁻¹. Firstly, the Raman spectra of myosin, Arg, Lys and His were obtained respectively. Secondly, we scanned the Raman spectra of the mixtures of myosin with amino acids, called myosin-Arg, myosin-Lys and myosin-His, respectively.

2.6. Statistical analysis

All data were calculated by three independent experiments and graphs were obtained using Origin 8.0 (OriginLab Co., Northampton, MA, USA), especially, the data and figures from Raman were performed by OMINIC 8.2 (Thermo Fisher Co. Ltd., Waltham, MA, USA) and the preserved data of 1600–1700 cm⁻¹ were performed by PeakFit 4.12 (Seasolve Software Inc. Framingham, MA, USA). The buffer was 0.1 M NaCl-20 mM Tris-HCl (pH 7.0).

3. Results and discussion

3.1. CD spectroscopy

Figure 1(a) shows two classical negative peaks near 208 and 222 nm, which symbolize the α-helix structure of native myosin (Cao & Xiong, 2015). Figure 1(b–d) imply that amino acid solutions had no negative bands near 208 or 222 nm and the CD values were independent on the concentrations of Lys, Arg or His after 235 nm. Furthermore, Figure 1(b,c) show a distinct regularity that the lower the concentration was, the closer to 0 near 222 nm the CD values were. For additive concentration less than 10 mM, the intrusion of Lys and Arg in the determination of secondary structures of fish myosin could be ignored due to their own minor values. However, Arg and Lys might disturb the result of secondary structures of myosin when the concentration added up to 20 mM because the CD values were up to 12.98 and 15.18 mdeg, respectively, demonstrating that the adequate additive concentration of Lys or Arg in CD method was less than 10 mM. Figure 1(d) shows disordered and confusing spectra from 200 to 250 nm except for 1 mM His, and the CD value of 1 mM His was not close to 0 near 222 nm, which may be resulted from the special structure of His.

Figure 2(a,b) show that Arg and Lys changed the CD values at 222 nm, demonstrating the α-helix content changed at different concentrations of Arg and Lys (Guo et al., 2015). As the α-helix structure of myosin was mainly stabilized by hydrogen bonds between the carbonyl oxygen and amino hydrogen of the polypeptide chain (Cao & Xiong, 2015; Liu et al., 2008), the addition the Arg and Lys may have disturbed these hydrogen bonds in the myosin molecule. Furthermore, the negative peak position of myosin added with 20 mM Arg or Lys shifted to the right and the minima became notably larger than those samples added with other concentrations whose negative peak values close each other, indicating that 20 mM Arg or Lys might disturb the determination of secondary structures of myosin and affect the actual CD values (Figures 2(a,b) and 1(b,c)). A shift of negative peak position was also
Figure 1. Espectros de CD de miosina y diferentes aminoácidos. (a) miosina, (b) Lys, (c) Arg, (d) His.

Figura 1. Espectros de CD de miosina y diferentes aminoácidos. (a) miosina, (b) Lys, (c) Arg, (d) His.

Figure 2. Espectros de CD de la solución de miosina añadida con diferentes aminoácidos. (a) miosina-Arg, (b) miosina-Lys, (c) miosina-His.

Figura 2. Espectros de CD de la solución de miosina añadida con diferentes aminoácidos. (a) miosina-Arg, (b) miosina-Lys, (c) miosina-His.
observed in the myosin samples added with His (Figure 2(c)). In addition, the CD spectra of myosin added with 5, 10 and 20 mM His were confusing and disordered, suggesting that CD method may be not suitable for the determination of secondary structures of myosin added with His (Figures 2(c) and 1(d)).

### 3.2. Raman spectroscopy

The amide I band (1600–1700 cm\(^{-1}\)) mainly involved C = O stretching, N-H in-plane bending, C-C-N vibration as well as C-N stretching vibration (Herrero, 2008; LiChan, 1996). It is generally admitted that the amide I band dropping within 1650–1658 cm\(^{-1}\) is ascribed to a high content of α-helix.
while the amide I band dropping within 1665–1680 cm$^{-1}$ is ascribed to a high content of β-sheet, and the band dropping within 1660–1665 cm$^{-1}$ is ascribed to a high content of random coil structures (Tuma, 2005). Figure 3(b) showed several peaks similar with myosin (Figure 3(a)) from 1650 cm$^{-1}$ to 1700 cm$^{-1}$. Figure 3(c) had weak scattering from 1650 cm$^{-1}$ to 1700 cm$^{-1}$ and Figure 3(d) had no scattering peak after 1650 cm$^{-1}$, which indicated that Raman method may be more applicable for the determination of Arg and His than Lys applied to myosin.

Figure 4 shows the evolution of Raman spectra of myosin samples added with different amino acids (1 mM). Figure 4 (a–c) denoted that Lys, His or Arg changed the classical scattering peaks of Raman spectroscopy of myosin, compared with the control (Figure 3(a)). However, Figure 4(a) showed more scattering peaks than Figure 4(b,c) between 1650 and 1700 cm$^{-1}$, which may be a consequence of the scattering of Lys itself shown in Figure 3(b), exhibiting a result that Raman method may be more suitable for the determination of Arg and His than Lys when they were added to myosin because the disturb of Lys must be taken into account.

4. Conclusions

His exhibits disordered and confusing spectra from 200 to 250 nm in CD spectroscopy and negligible scattering from 1650 cm$^{-1}$ to 1700 cm$^{-1}$ in Raman spectroscopy, indicating that Raman method may be more applicable to myosin added with His than CD spectroscopy method. Lys shows several scattering peaks from 1650 cm$^{-1}$ to 1700 cm$^{-1}$ in Raman spectroscopy while no negative bands near 208 or 222 nm in CD spectra, demonstrating that CD method maybe more applicable to myosin added with Lys than Raman spectroscopy method, while the adequate concentration of Lys should be under 10 mM. Furthermore, both CD and Raman methods can be applied to myosin added with Arg, however, the latter is recommended and the adequate additive content of Arg in CD method should be less than 10 mM.

Disclosure statement

No potential conflict of interest was reported by the authors.

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