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Interaction between Lipid and Protein

IV. The adaptation of the ultraviolet absorption spectrum method for the determination of protein contents in the mixtures of lipids and myosin B

Sei-ichi ANDOU*, Kōzō TAKAMA** and Kōichi ZAMA**

Abstract

An attempt was made to determine the protein contents in the mixtures of lipids and myosin B by means of the ultraviolet absorption spectrum method.

In the case of the constant of lecithin concentration with varying myosin B one, the absorbances at 278 nm and 265 nm of the mixtures showed the sum of those of myosin B and lecithin. The similar results were also observed in the case of the constant of myosin B concentration with varying lecithin one. Also, it was recognized that this relationship was not influenced by the kinds of lipids. Consequently, in the case of the known lecithin concentration, the protein contents in the mixtures of lipids and myosin B might be determined from the differences in the absorbances of the mixtures and lecithin at 278 nm.

Also, in the case of the indefinite lecithin concentration, the protein contents in the mixtures of lipids and myosin B were simply determined by the following equation,

\[ P_{278} = 6.379M_{278} - 5.798M_{265} + 0.075 \]

where \( P_{278} \) is the absorbance of myosin B at 278 nm, and \( M_{278} \) and \( M_{265} \) are the absorbances of the mixtures of lipids and myosin B at 278 nm and 265 nm, respectively.

Taking into consideration the advantage that the sample can be recovered as it is, it is presumed that the ultraviolet absorption spectrum method is evaluated in the studies on the interactions between lipids and fish muscle proteins.

Introduction

The Lowry method\(^1\), the biuret method\(^2\) and the ultraviolet absorption spectrum method\(^3\) are generally used in the determinations of the protein contents. These methods are useful because of the rapid determinations of the protein contents, but the methods are lacking in accuracy if the turbid solutions are applied.

It has been reported that the myosin B prepared from fish muscle is frequently contaminated by the lipids\(^4\)–\(^7\). Consequently, the difficulties are liable to occur in the determinations of fish muscle protein contents. Therefore, the establishment of the quantitative method of fish muscle proteins containing the lipids is necessary in order to study the interactions between lipids and proteins.

The present investigation was carried out to establish the simplified quantitative
method for the determinations of the protein contents in the myosin B solution containing lipids by means of the ultraviolet absorption spectrum method. 3)

**Materials and Methods**

Myosin B was prepared from the dorsal muscle of rainbow trout (Salmo gairdneri irideus GIBBONS) as described by Konno et al. 8)

Purified lecithin prepared from northern blenny roe and fatty acid methylester prepared from squid oil were used as the polar lipid and non-polar lipid, respectively.

The preparation of myosin B and mixed lipid system was carried out as follows. A peroxide free diethyl ether solution containing an aliquot amount of lipid was added to a test tube. The ether was evaporated using a flow of nitrogen gas. Then 5 ml aliquot of myosin B solution was added to the test tube, and was mixed by an automixer three times for 15 seconds with a 5 second interval between each mixing.

The protein contents in the mixtures of lipids and myosin B were determined by an ultraviolet absorption spectrum method. 3) The determination of the ultraviolet absorption spectrum was carried out by using a Hitachi 556 double wavelength spectrophotometer. Bovine serum albumin fraction V was purchased from Wako Pure Chem. Ind. Ltd. and used for the standard protein in the ultraviolet absorption spectrum method.

**Results**

Fig. 1 shows the ultraviolet absorption spectra of myosin B solution (0.6 M KCl-phosphate buffer, pH 7.2), lecithin and fatty acid methylester suspensions in the 0.6 M KCl-phosphate buffer solution (pH 7.2). The maximum and minimum absorptions were shown at 278 nm and 250 nm in myosin B solution, respectively. On the other hand, the maximum and minimum absorptions were not found in

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Fig. 1. The ultraviolet absorption spectra of myosin B solution (0.6 M KCl-phosphate buffer, pH 7.2), lecithin and fatty acid methylester suspensions in the 0.6 M KCl-phosphate buffer solution (pH 7.2).

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lecithin and fatty acid methylester suspensions.

Fig. 2 shows the ultraviolet absorption spectra of myosin B solution and lecithin suspension added to the myosin B solution. The maximum ($E_{\text{max}}$) and minimum absorbances ($E_{\text{min}}$) of MB-L were higher compared with those of myosin B solution because of the absorbance of lecithin suspension. The ratios of $E_{\text{min}}$ to $E_{\text{max}}$ enlarged by increasing the amount of lecithin. Also, it was found that $E_{\text{min}}$ shifted from 250 nm to 265 nm by increasing the amount of lecithin.

Fig. 3 shows the relationships between the absorbances at 278 nm ($E_{278}$) and protein concentration in myosin B solution and lecithin suspension added to the myosin B solution (MB-L). The data of MB-L were parallel to those of myosin B solution, when the amount of lecithin added was constant (0.4 mg/ml).

Fig. 4 shows the relationships between $E_{278}$ and lecithin concentration in lecithin suspension and MB-L. The data of MB-L were parallel to those of lecithin suspension, when the amount of myosin B added was constant (0.8 mg/ml).

Fig. 5 shows the relationships between the absorbances at 265 nm ($E_{265}$) and protein concentration in myosin B solution and MB-L. $E_{265}$ of MB-L ran parallel to that of myosin B solution, if lecithin concentration was constant (0.4 mg/ml).
Fig. 5. The relationships between the absorbances at 265 nm ($E_{265}$) and protein concentration in myosin B solution and lecithin suspension added to the myosin B solution (MB-L).

Fig. 6 shows the relationships between $E_{265}$ and lecithin concentration in lecithin suspension and MB-L. $E_{265}$ of MB-L ran parallel to that of the lecithin suspension, if myosin B concentration was constant (0.8 mg/ml).

The data shown in Figs. 3, 4, 5 and 6 implied that $E_{278}$ and $E_{266}$ of MB-L were simply based on the sum of those absorbances of myosin B solution and lecithin suspension. Also, when the mixtures of lecithin and fatty acid methylester were added to myosin B solution, the similar results as MB-L were shown.

**Discussion**

In the case of the known lecithin concentration, Fig. 3 implied that protein contents in MB-L might be determined from the differences in the absorbances of MB-L and lecithin suspension at 278 nm.

However, lecithin concentration in myosin B preparation is indefinite in practice. Consequently, protein contents in MB-L are not determined directly from the above idea.
Now, the following assumptions are proposed, as shown in Fig. 7.

\[ M_{265} - P_{265} = F \cdot L_{265} \]  
\[ M_{278} - P_{278} = F' \cdot L_{278} \]

where \( M_{265}, P_{265}, \) and \( L_{265} \) are \( E_{265} \) of MB-L, myosin B solution and lecithin suspension, respectively, and \( M_{278}, P_{278}, \) and \( L_{278} \) are \( E_{278} \) of MB-L, myosin B solution and lecithin suspension, respectively. Also, Fig. 8 shows the relationships between \( E_{265} \) and \( E_{278} \) of myosin B solution. Fig. 9 shows the relationships between \( E_{265} \) and \( E_{278} \) of lecithin suspension. The equation (3) was derived from the equation (1) and (2), and Figs. 8 and 9.

\[ P_{278} = \frac{F \cdot M_{278} - 0.924 \cdot F' \cdot M_{265} + F' \cdot (9.24 \cdot 10^{-4} + 0.011 \cdot F)}{F - 0.795 \cdot F'} \]  
(3)

\( F \) and \( F' \) were calculated from the experimental data which were applied to the equations (1) and (2). The equation (4) was derived from the equation (3).

\[ P_{278} = 6.523 \cdot M_{278} - 5.928 \cdot M_{265} + 0.077 \]  
(4)

In order to equalize myosin B concentration derived from the equation (4) to the true concentration, another factor was introduced into the equation (4), and the equation (5) was formed.

\[ P_{278} = 6.379 \cdot M_{278} - 5.798 \cdot M_{265} + 0.075 \]  
(5)

Equation (5) was generally applicable for determining the protein contents in the myosin B preparation which were contaminated by lipids. Furthermore, taking into consideration the advantage that the sample can be recovered as it is, it is presumed that the ultraviolet absorption spectrum method is evaluated in the studies on the interactions between lipids and proteins.

References

ANDOU et al.: Protein measurement by UV spectrum method


