Bull. Fac. Fish. Hokkaido Univ.
32(2), 188-193. 1981.

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, $P_{278}=6.379\cdot M_{278}-5.798\cdot M_{265}+0.075$, 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

* *Course of Nutritional Science, Hakodate Junior College*
(函館短期大学食物栄養学科)
** *Laboratory of Food Chemistry I, Faculty of Fisheries, Hokkaido University*
(北海道大学水産学部食品化学第一講座)

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

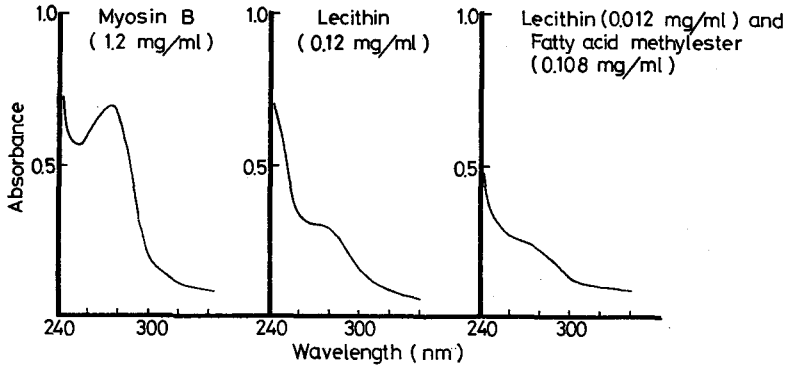

Fig. 1. The ultraviolet absorption spectra of myosin B solution (0.6 M KCl-phosphate buffer, pH 7.2), lecithin and fatty acid methylester suspensions in the 0.6 M KCl-phosphate buffer solution (pH 7.2).

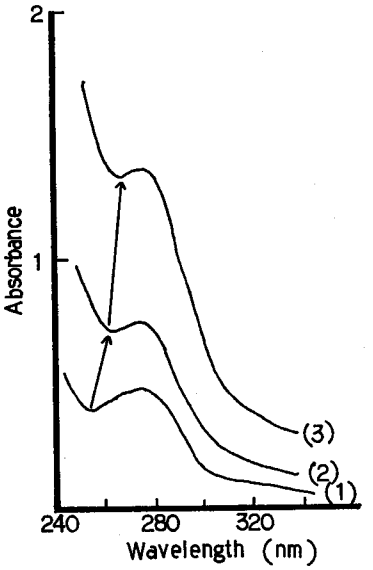

Fig. 2. The ultraviolet absorption spectra of myosin B solution and lecithin suspension added to the myosin B solution.
(1) Myosin B (0.74 mg/ml)
(2) Myosin B (0.74 mg/ml) +Lecithin (0.12 mg/ml)
(3) Myosin B (0.74 mg/ml) +Lecithin (0.37 mg/ml)

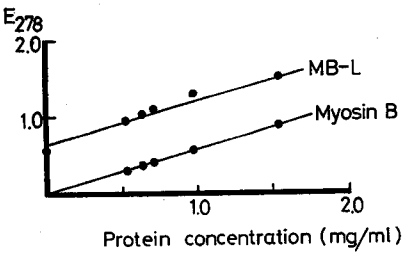

Fig. 3. The relationships between the absorbances at 278 nm (E_{278}) and protein concentration in myosin B solution and lecithin suspension added to the myosin B solution (MB-L).

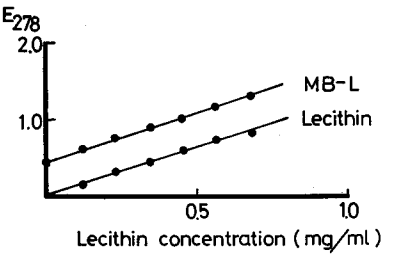

Fig. 4. The relationships between the absorbances at 278 nm (E_{278}) and lecithin concentration in lecithin suspension and the lecithin suspension added to myosin B solution (MB-L).

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 (MB-L). The maximum (E_{max}) and minimum absorbances (E_{min}) of MB-L were higher compared with those of myosin B solution because of the absorbance of lecithin suspension. The ratios of E_{min} to E_{max} enlarged by increasing the amount of lecithin. Also, it was found that E_{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 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).

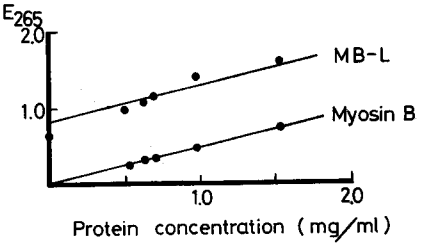

Fig. 5. The relationships between the absorbances at 265 nm (E_{265}) and protein concentration in myosin B solution and lecithin suspension added to the myosin B solution (MB-L).

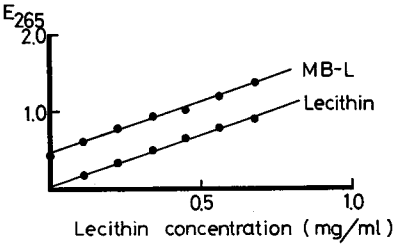

Fig. 6. The relationships between the absorbances at 265 nm (E_{265}) and lecithin concentration in lecithin suspension and the lecithin suspension added to myosin B solution (MB-L).

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_{265} 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 mysoin 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.

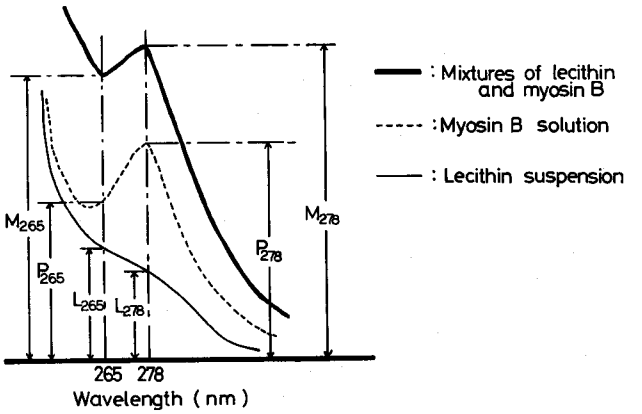

Fig. 7. Schematic representation of the ultraviolet absorption spectra of myosin B solution, lecithin suspension and the mixtures of lecithin and myosin B.

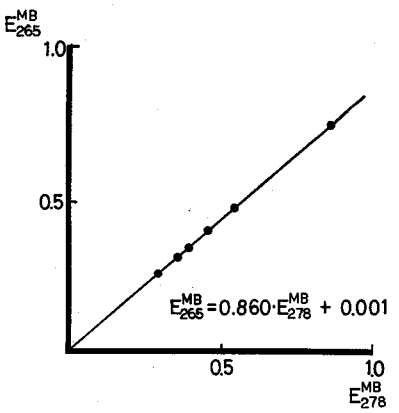

Fig. 8. The relationships between the absorbance at 278 nm (E^{MB}_{278}) and that at 265nm (E^{MB}_{265}) of myosin B solution.

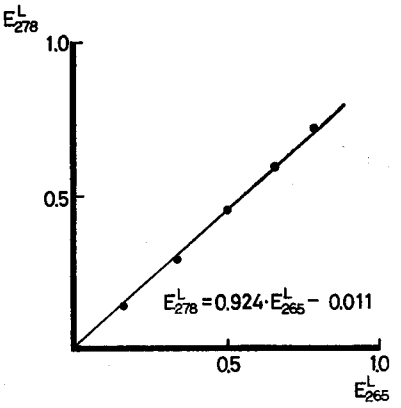

Fig. 9. The relationships between the absorbance at 278 nm (E^{L}_{278}) and that at 265 nm (E^{L}_{265}) of lecithin suspension.

Now, the following assumptions are proposed, as shown in Fig. 7.

$$M_{265}-P_{265}=F\cdot L_{265} \quad (1)$$

$$M_{278}-P_{278}=F'\cdot L_{278} \quad (2)$$

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.

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

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).

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

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.

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

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

1) Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measure-

ment with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.
2) Gornall, A.G., Bardawill, C.J. and David, M.M. (1949). Determination of serum proteins by means of the biuret reaction. *Ibid.* **177**, 751–766.
3) Sugawara, K. and Soejima, M. (1979). Seibutsukagakujikkenhō. Tanpakushitsu no teiryōhō. 203p. Gakkai shuppan sentā, Tokyo, Japan. (In Japanese).
4) Ikeda, S. and Taguchi, T. (1967). Protective effect of α-tocopherol on the solubility of actomyosin from yellowtail muscle. *Bull. Japan. Soc. Sci. Fish.* **33**, 567–571.
5) Taguchi, T. and Ikeda, S. (1968). Studies on the properties of fish actomyosin – III. Lecithin found in the actomyosin from fish muscle. *Ibid.* **34**, 339–343.
6) Taguchi, T. (1973). Lecithin residues in carp muscle myosin B and its ATPase activity. *Ibid.* **39**, 969–972.
7) Hamada, I., Tsuji, K., Nakayama, T. and Niwa, E. (1977). Oxidative denaturation of actomyosin. *Ibid.* **43**, 1105–1109. (In Japanese with English abstract).
8) Konno, K. and Seki, N. (1974). Calcium desensitized actomyosin from carp muscle. *Ibid.* **40**, 1277–1284. (In Japanese with English abstract).