Studies on the Egg White Protein

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Introduction

The fraction of egg white soluble in halfsaturated ammonium sulphate solution contains in addition to a crystallizable ovalbumin another noncrystallizable albumin which is more soluble in ammonium sulphate solutions. Osborn and Campbell[1] observed a difference in rotation and in sulphur content of the crystalline ovalbumin and the more soluble amorphous fractions and termed the amorphous albumin 'conalbumin'. Subsequent attempts at purification of this protein did not lead to the isolation of a pure prouct, since the preparation always contained ovalbumin. Antisera to the purified conalbumin preparation always reacted with crystalline ovalbumin, indicating contamination with this protein[2].

Since 1940 Longsworth[3], Alderton[4], Deutsch[5], Forsythe[6], and Weber[7] have studied on the isolation and electrophoretic behaviors of conalbumin. Longsworth and Alderton recognized that the conalbumin, which was prepared by them, consists of two components by means of electrophoretic and ultracentrifugal analysis. Deutsch and Forsythe separated a conalbumin from egg white protein by alcohol fractionation procedure and recognized that the conalbumin consists of homogeneous monocomponent electrophoretically. But all of these conalbumin, prepared by the above investigators, were not obtained in crystalline form. In 1951 Warner and Weber reported the isolation of conalbumin in crystalline form both as the ferric iron complex and the iron free protein.

Bain and Deutsch reported that the conalbumin prepared by them regulaly contains flavin and offered the question as to whether the flavin is combined with the protein to form a flavoprotein. Forsythe and Foster similaly found that the crude conalbumin fraction contains all the yellow color of egg white. On further fractionation, they obtained two fractions and one of them contains the greater part of the conalbumin with no color, while the other fraction, which is water insoluble but soluble in 5% NaCl, contains all the yellow color of egg white. No further data on this fraction were given, but it seems possible that this may be a conjugate of protein and flavin. Until today any explanation has not been given on the problem with what kind of protein the flavin is combined.

In the present paper the author shows the isolation of the conalbumin from egg white by Alderton's method and then indicates that flavin of egg white does not combine with conalbumin but it combines with ovomucoid.

Experiment and Discussion

1. On the isolation of conalbumin

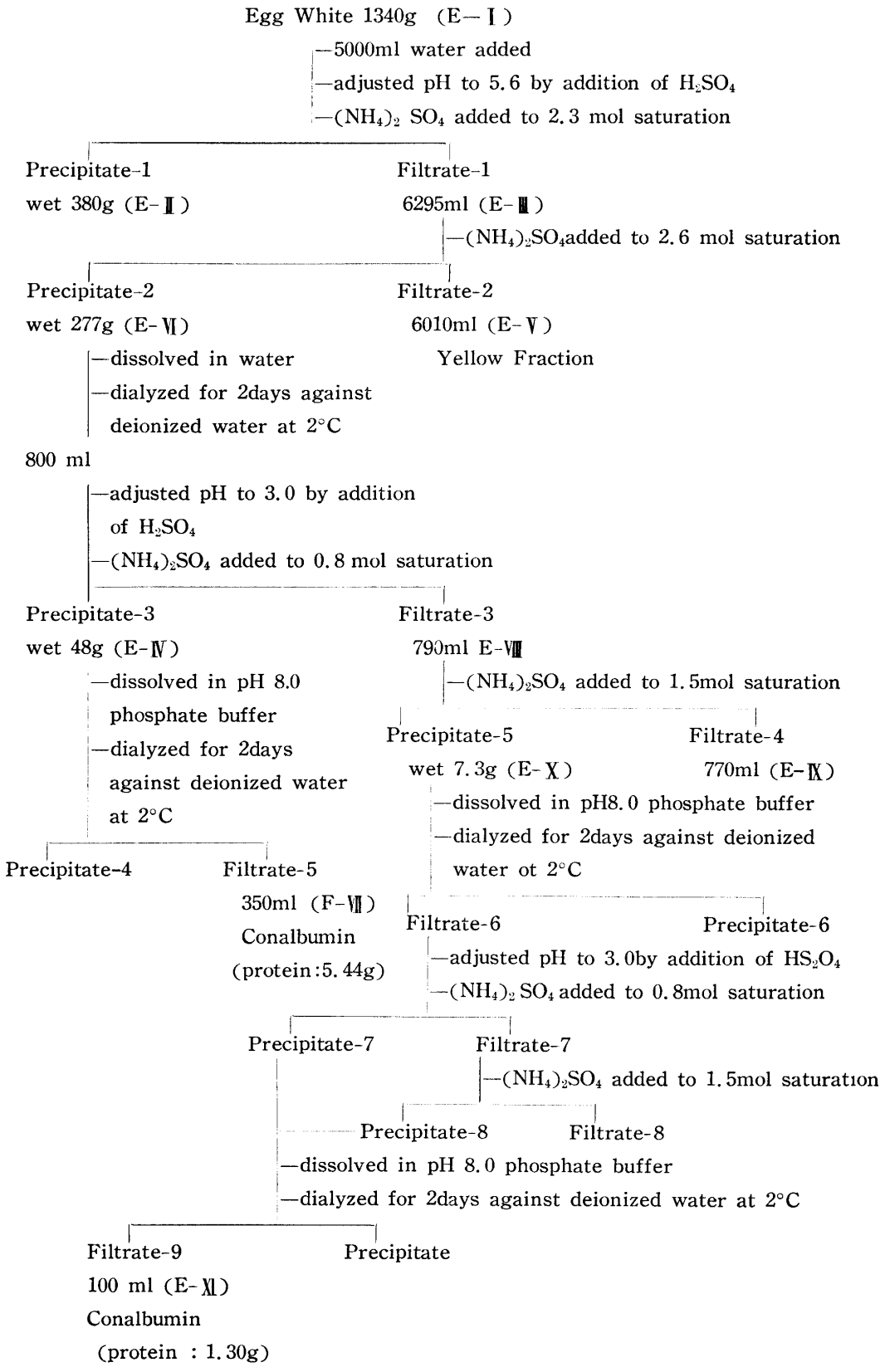
Fresh eggs were secured from the Poultry Farm of the Saikyo University and the whites were separated from the yolk and blended in a waring blender. The author isolated the conalbumin from these whites by the Alderton's procedure as shown in Table-1.

The electrophoretic analysis of the fractions, which were obtained during isolation of the conalbumin, was carried out by Hitachi- Tiselius Apparatus. The electrophoretic patterns of each fraction are shown in Fig-1.

From these electrophoretic patterns (E-Ⅶ and E-Ⅺ) and fractionation scheme mentioned above, it may be concluded that the conalbumin is to be precipitated from 0.8mol to 1.5mol saturation of ammonium sulphate and that its electrophoretic pattern shows homogeneous single boundary.

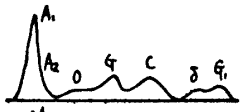
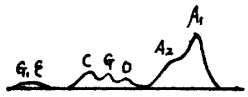
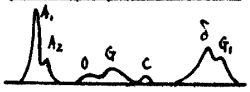
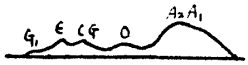
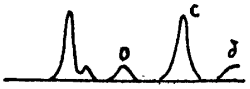
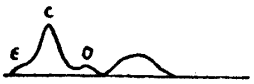
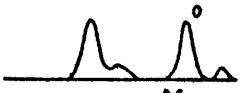
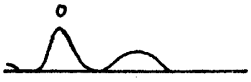
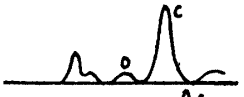
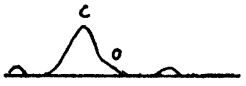
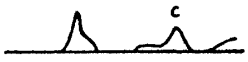
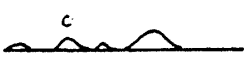
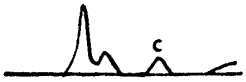
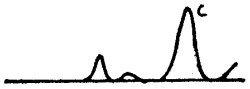
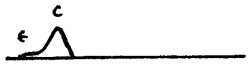
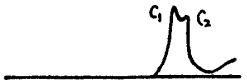
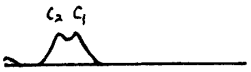
Longsworth and Alderton's conalbumin consisted of two components and it showed varying electrophoretic patterns according to the acid modification, but the conalbumin preparation,

Table 1 Isolation of Conalbumin



which was isolated by Bain and Deutsch's alcohol fractionation, showed only one component electrophoretically.

During the isolation procedure, as the author treated the conalbumin for a short time as possible at acid pH values, the author's conalbumin did not modify by acid. But as shown in the pattern E-Ⅻ after dialysis for three days at pH 3.0, the

Fraction	Ascending ←	Descending →	Potential gradient. (Volt, Cm^{-1})	Buffer	Sec.
E—I			7.1	Phosphate buffer $\Gamma/2=0.1$ pH=8.0	5400
E—II			7.3	〃	〃
E—III			7.2	〃	〃
E—IV			7.5	〃	〃
E—V			7.2	〃	〃
E—VI			7.9	〃	〃
E—VII			7.2	〃	〃
E—VIII			7.6	〃	〃
E—IX			6.8	〃	〃
E—X			8.8	〃	〃
E—XI			6.7	〃	6000
E—XII			6.6	〃	〃

A_1 A_2·····albumin O·····ovomucoid G G_1·····globulin C·····conalbumin

Fig. 1 Electrophoretic Patterns of Each Fraction

author 's conalbumin as well as Longsworth and Alderton 's conalbumin is] converted to two components (C_1 'and C_2) electrophoretically by acid modification.

About modification of conalbumin, Longsworth recognized that if the dialysis was carried out at pH 7.54, only a small amount of C_2 (modified conalbumin) was present, if the dialysis carried out below pH 7.54, the proportion of C_1 (native conalbumin) and C_2 varied depending on the pH, C_2 being in the stable form below pH 4.0, and C_1 being in the stable form at pH 7. 54, as shown in Fig-2.[3]

But from the above experiments and other experiments on modification of conalbumin, the author did not recognize Longsworth's experiment described above, and agreed with Phelp's[8] experiment on the acid modification of conalbumin.

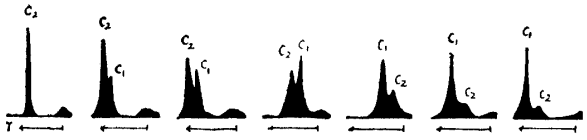


Fig. 2 Electrophoretic patterns illustrating the complexity of conalbumin as prepared from egg white protein.

2. On the relation between conalbumin and yellow color of egg white.

As shown in the Table-1, all yellow color of egg white migrated into fraction Fil-2 and

conalbumin was not contained in this fraction, therefore it may be concluded that conalbumin has no relation to yellow color of egg white.

Fil-2 fraction was dialyzed for 2 days against several buffers such as pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0. At pH 2.0, 3.0, and 4.0 yellow color of Fil-2 fraction is migrated on into the outside of cellophane membrane to Donnan's membrane equilibrium. But at pH 5.0 and 6.0 yellow color could not be recognized with the naked eye at the outside of cellophane, but the fluorescence of its color could be recognized under the ultra-violet lamp. In the case of pH 7.0 and 8.0, both yellow color and its fluorescence could not be recognized at the outside of membrane.

From the above experiments, it may be apparent that yellow color can easily be released from protein which is combined with its color, at acid side below pH 4.0, but it is combined tightly with protein at alkaline side above pH 7.0. Then yellow color containing protein was dialyzed against pH 3.0 buffer and the dialyzed yellow color, having been collected and concentrated, was analyzed by spectrophotometric and paperchromatographic method. From these experiments the author recognized that yellow color is riboflavin as Deutsch has described.
The protein, remained in cellophane membrane after dialysis, is capable to combine with riboflavin, flavinmononucleotide and yellow color which is released from yellow protein, and each mixtures was dialyzed for 2 days respectively at 2°C against pH 8.0, phosphate buffer and tapping deionized water, but riboflavin, flavinmononuclectide and yellow color of each mixtures did not release from cellophane membrane, and the flavoprotein, which is thus combined with them, remained in membrane.

In 1948 Bain and Deutsch showed that the riboflavin is bound to the conalbumin but it can be removed by dialysis only on the acid side of the isoelectric point and then Forsythe and Foster (1950) recognized that all of the yellow color of the egg white was in the crude conalbumin fraction but further fractionation of it, it was resulted in two main fractions, and one of them contained a cosiderably higher proportion of conalbumin and the other, insoluble in water but soluble in 5% NaCl, cotained all of the yellow constituent which had been originally present in the fraction, and concluded that the riboflavin is not bound to the characteristic conalbumin constituent, and that the bound riboflavin appreciably alters the solubility properties of coalbumin.

From these experiments, the author came to disapprove the above described experimental results by Bain, Deutsch, Forsythe and Foster, and recognized that the protein moiety of yellow protein is liable to combine with those of riboflavin, flavinmononucleotide and yellow color, and also that conalbumin and ovalbumin have no liability to combine with them by the same experiments, therefore it may be concluded that the protein moiety of yellow protein, is not a conalbumin.

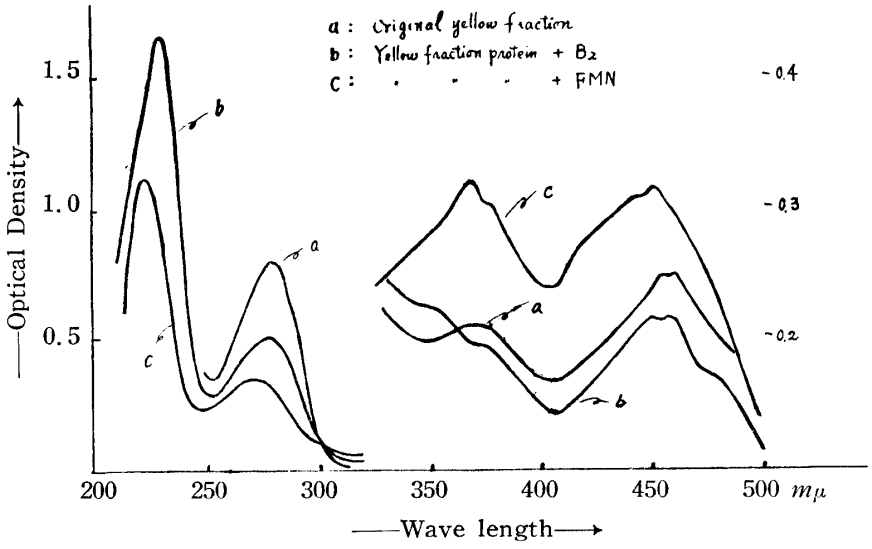


Fig. 3 Spectrum of yellow fraction, yellow fraction protein + B_2 and yellow fraction protein + FMN.

The absorption curves of the original yellow color pro-

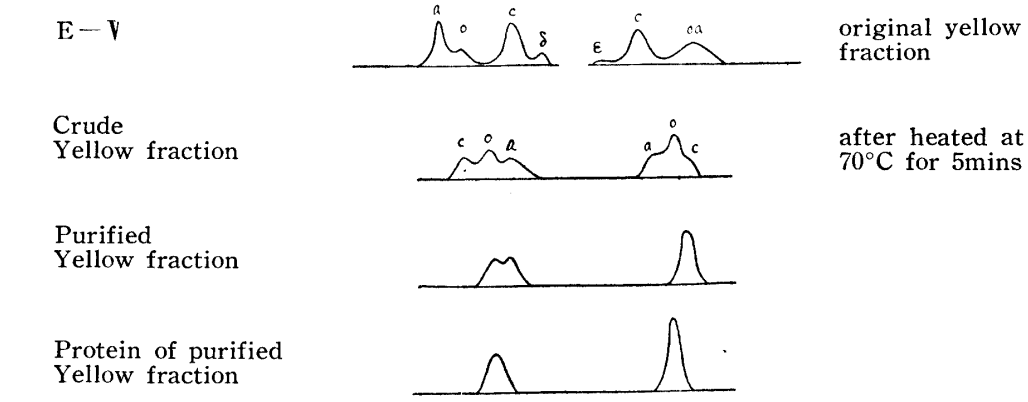
Table 2 Quantity of protein and riboflavin in yellow fration and yellow fraction protein+B_2 and FMN.

	Protein mg per cc	Riboflavin γ per cc	Riboflavin/Protein mol ratio
Original yellow fraction	10.19	3.14	0.022
Protein of yellow fraction+B_2	7.25	10.93	0.108
〃 +FMN	0.50	7.13	1.069

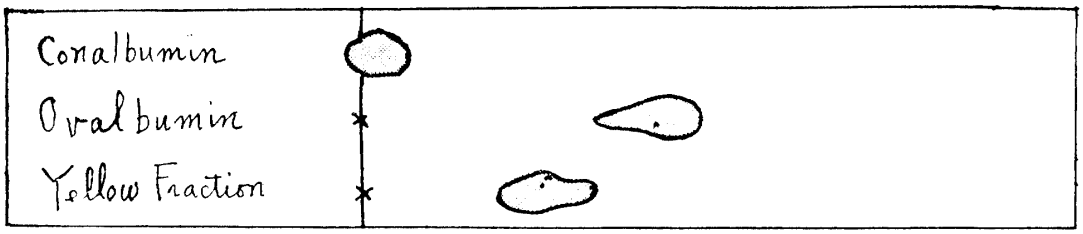
tein and the synthetic flavoproteins, which contained riboflavin or flavinmononucleotide besides the protein moiety of original yellow color protein, are shown in Fig-3, and their quantity of protein and riboflavin are also shown in Table-2.

As shown in Fig-3, the original yellow color protein (curve a) exhibits the absorption maxima at 445, 375 and 280mμ, and the flavoprotein, when combined with flavinmononucleotide, exhibits the maxima at 450, 370, 280 and 220 mμ, and when combined with riboflavin exhibits the maxima at 450, 375, 360, 280 and 230 mμ.

From these absorption spectrum the author did not come to the conclusion whether the yellow color is flavinmononucleotide or riboflavin, moreover this conclusion was confirmed by the two florescent spots (riboflavin, flavinmononucleotide and flavinadeninedinucleotide)on paperchromatogram under ultraviolet lamp. As shown in E-V pattern of Fig-1, Fil-2 fraction is composed of ovomucoid, ovalbumin A_1, and A_2 and it is clear by the above described experiments, both ovalbumin A_1 and A_2 have not a capability of combination with riboflavin and its derivatives, therefore it may be concluded that ovomucoid has a capability to combine with riboflavin and its derivatives. If the molecular weight of ovomucoid is 27000, molecular ratio of ovomucoid and riboflavin in original yellow protein and



electrophoretic condition : pH 8.0 phosphate buffer (Γ/2=0.1)
4°C, potential gradient 3.7 Volt Cm^{-1}
mobitity of yellow fraction protein $=-4.5\times10^{-5}$ Cm^2, $Volt^{-1}$, Sec^{-1}



paperelectrophoretic condition : pH 8.6 Veronal buffer (Γ/2=0.045)
250 Volt, 0.6 mA, colored by BPB.

Fig. 4 Electrophoretic and paperelectrophoretic patterns of yellow fraction.

synthetic flavoproteins, are respectively 0.022, 0.108 and 1.069 as shown in Table-2. And it may be supposed that the difference of combination ratio between riboflavin and flavinmononucleotide depends on their solubility. The precipitate, which had been produced after heating Fil-2 fraction (E-V pattern) at 70°C for 5 mins, was filtered off and then the filtrate was adjusted pH to 5.6 and saturated with ammonium sulphate upto 3mol saturation, and the precipitate was filtered off. The filtrate thus obtained was heated again at 70°C for 5 mins and the precipitate was filtered off and the above treatment was repeated. The filtrate was pure yellow protein fraction and its electrophoretic pattern and paperelectrophoretic patteern are shown in Fig-4.

As shown in Fig-4, the purified yellow fraction consisted of one component as in ascending pattern, but it is convered into two components as showing in descending pattern, and also the same results as shown in electrophoresis have been recognized in paperelectrophoresis. Above phenomena will be explained as typical interaction between yellow color and protein From the mobility of the protein of purified yellow fraction ($-4.5X10^{-5}$ Cm^2, Vol^{-1}, Sec^{-1}) and its chemical properties it may be concluded that its protein moiety is ovomucoid.

Table-3 Molecular extinction coefficient of riboflavin, yellow fraction and yellow fraction protein + riboflavine.

mμ	Riboflavine $mol^{-1}cm^{-1} \times 10^{-3}$	Yellow fraction $mol^{-1}cm^{-1} \times 10^{-3}$	Yellow fraction protein B $mol^{-1}cm^{-1} \times 10^{-3}$
510	0.4	—	—
450	12.2	0.335	1.120
375	10.6	0.341	0.980
260	27.7	—	—

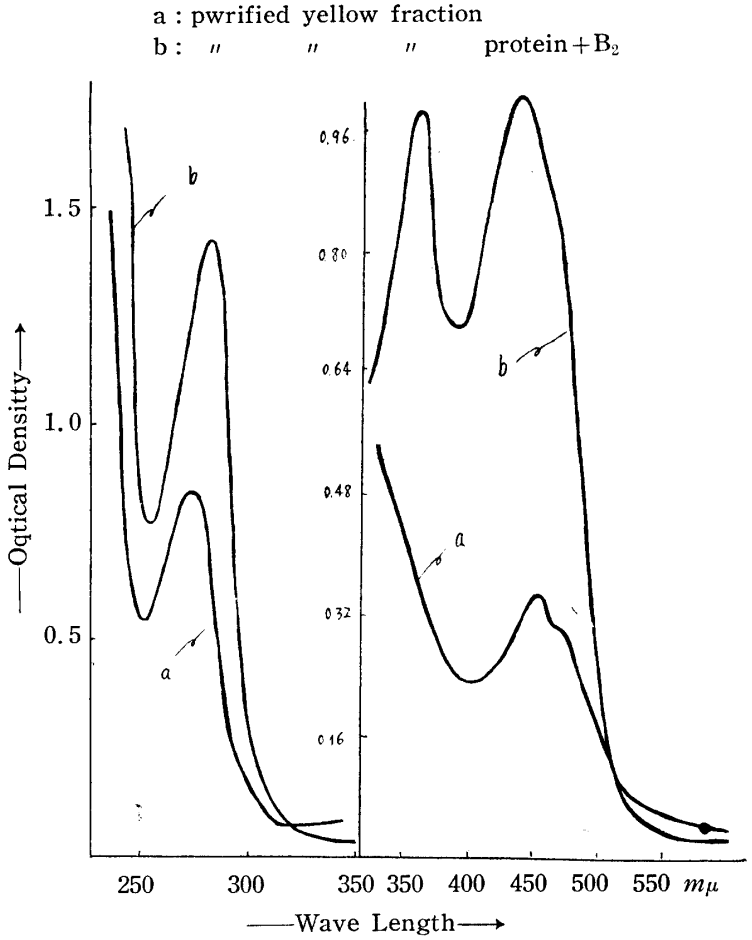


Fig. 5 Spectrum of purified yellow fraction and yellow fraction protein+B_2

The riboflavin, which is dissolved in pH 8.0 phosphate buffer, was added to the above protein moiety of yellow fraction (ovomucoid) and then dialyzed for 2 days against pH 8.0 phosphate buffer and tapping deionized water. The spectrums of purified yellow fraction and above artificial yellow color fraction and the combination ratio of protein and yellow color of them are shown in Fig-5 and Table-3. In the purified yellow fraction and artificial yellow fraction, the combination ratio of protein and yellow color which was calculated from Table-3, was 1 mol: 1 mol and 1 mol: 4 mol respectively. Then the author analyzed the yellow color of purified yellow fraction by the procedure of paperchromatography using two solvents such as lutidine and benzylalcohol and decided that its flavin color is riboflavin. From the fact that total riboflavin of egg white is about 0.45 mg% of

which 3 to 5% is flavinmononucleotide and 45 to 50% are flavinadeninedinucleotide and riboflavin respetctively, and also from the previous experiment of crude yellow fraction the author detected flavinmononucleotide as yellow color. The author supposes that yellow color is not always only riboflavin and the further investigation on this problem is necessary and important.

Summary

1. The author studied that the electrophoretic analysis of whole egg white protein by the ammonium sulphate fractionation procedure, and recognized that the conalbumin, which is prepared by the Alderton 's method gave electrophoretic homogeneous pattern, but after dialysis for 3 days at acid pH values it was modified to two components electrophoretically.

2. The isolated conalbumin was colorelss and did not combine with yellow color (riboflavin, flavinmononucleotide and flavinadeninedinucleotide) of egg white.

3. The yellow protein of egg white was concentrated into the portion which was precipitated by 2.6 mol saturation of ammonium sulphate and yellow color was released easily from the yellow protein at acid pH values (below pH 4.0) and combined tightly with its protein portion at alkalline pH value.

4. The yellow color of yellow protein in egg white was mainly riboflavin, and its protein moiety was ovomucoid.

5. The purified yellow fraction contained ovomucoid and riboflavin in a ratio 1 mol:1 mol and the protein moiety of yellow fraction (ovomucoid) had a capability of combination with riboflavin in a maxima ratio of 1 mol : 4 mol.

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要　　約

金 森 正 雄

1. 卵白蛋白を硫安分別法によつて分別して，その電気泳動分析を行つて，Alberton の方法で調製したコンアルブミンは電気泳動的に均一の泳動図を示すが，これを3日間酸性側で透析すると2成分に変化することをみとめた.

2. 単離コンアルブミンは無色で，卵白中の黄色色素（フラビン，FMN，FAD）とは結合しない.

3. 卵白中の黄色色素蛋白は卵白の硫安 2.6 mol 飽和溶液中に集中して存在し，その黄色色素は pH4.0 以下の酸性側で蛋白から容易にはなれ，アルカリ側で蛋白と堅く結合している.

4. 卵白の黄色色素蛋白の黄色色素はリボフラビンであり，その蛋白部分はオボムコイドである.

5. 純粋にした卵白の黄色色素蛋白はオボムコイドとリボフラビンとを 1mol : 1mol の比で含み，黄色色素蛋白の蛋白部分即ちオボムコイドはリボフラビンと最高 1mol : 4mol の比で結合する.