

Comparative Analysis of the Amphibian Blood Serum Protein by Electrophoresis

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電氣泳動法에 의한 兩棲類 血清蛋白質의 比較分析

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摘 要

兩棲類 五種 *Bombina orientalis*, *Cacopoides borealis*, *Bufo bufo asiaticus*, *Rana nigromaculata nigromaculata*, *Rana rugosa*의 血清蛋白質을 Paper electrophoresis의 方法으로 分析比較 하였다. 그 結果 *Bombina orientalis*의 血清蛋白質은 人間의 것과 마찬가지로 5個의 protein fraction으로 나누어지고 그중 fraction III蛋白質이 가장 많은 含量을 構成하며 peak가 가장 높다. *Cacopoides borealis*와 *Bufo bufo asiaticus*의 血清蛋白質은 4個의 protein fraction으로 나누어 지는데 *Cacopoides borealis*에서는 fraction IV protein이, *Bufo bufo asiaticus*에서는 fraction I protein이 가장 많은 含量을 構成하고 있다.

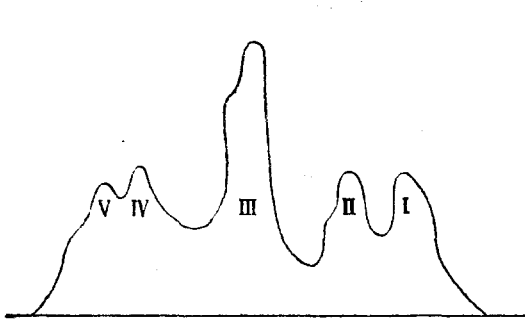
*Rana nigromaculata nigromaculata*와 *Rana rugosa*는 graph上에 나타난 peak로 보면 相互類似 하나 protein fraction數는 差異가 있다. 그리고 albumin은 全含量 percentage의 約半을 차지하고 있다. 即 種間의 距離가 멀수록 protein fraction의 差異가 percentage 含量에 있어서 더욱 明白하여짐을 暗示하여 준다.

INTRODUCTION

Several workers have shown that the electrophoretic pattern of blood serum protein are characteristic for different species (Moore, 1945; Deutsch and Goodloe, 1945; Lewis, Green and Page, 1952; Morris and Courtice, 1955). Deutsh (1945) studied the blood serum of human, rat, chicken and dog by electrophoresis. Deutsch and McShan (1949) made reports of their investigation on the plasma protein of some species of fish, turtle, snail and bull frog including snake and Mooris (1959) studied the proteins and lipids in the plasma of some species of australian fresh and salt fishes. This report is of the results of investigation at the field of taxonomical physiology on the electrophoretic patterns and relative quantities of the blood serum protein of amphibian such as *Bombina orientalis*, *Cacopoides borealis*, *Bufo bufo asiaticus*, *Rana nigromaculata nigromaculata* and *Rana rugosa* in Korea.

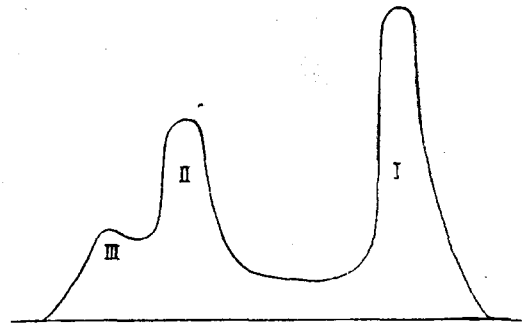
MATERIALS and METHODS

Five amphibian species were collected at some places near Seoul city in summer for the experimental materials. These animals were kept in temperature of $25 \pm 2^{\circ}\text{C}$ for a week without food so as to prevent the change of the blood serum protein by food and other particular conditions. Blood was obtained by cardiac puncture from each animal while they were alive. The blood samples were centrifuged as soon as possible after collection and the plasmas were then stored in the method of filter paper electrophoresis. A barbitrate buffer of pH 8.5, ionic strength 0.06M and Whatman No.1 chromatographic paper were adopted and the separations were carried out for 16 hours. The electric power used at the time was 110 volts and 1.2 amperes. The air bubbles contained in the filter paper strip were extracted by after the vacume pump. The blood serum protein (0.02ml) applied in the start line of the moisture filter paper strip and after it was laid in the paper electrophoresis for about 10 minutes.



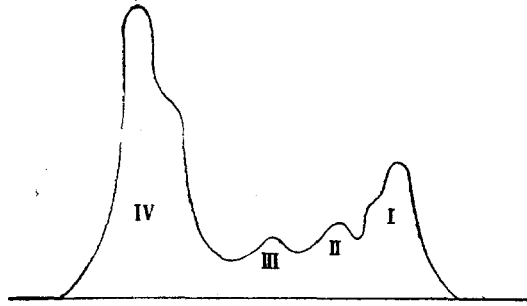
Fraction number	I	II	III	IV	V
Percentage composition	22.17 ±5.4	21.69 ±5.07	27.64 ±0.5	15.85 ±4.1	13.35 ±6.05

Fig. 1. Electrophoretic diagram of blood serum protein in *Bombina orientalis*.



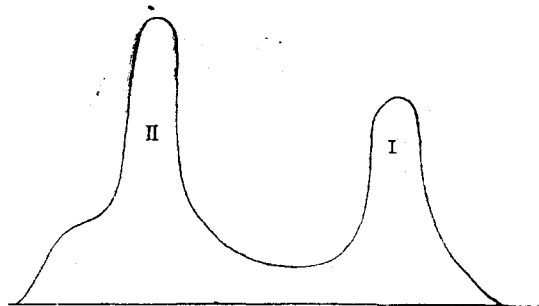
Fraction number	I	II	III
Percentage composition	10.86 ±2.43	45.40 ±4.67	43.74 ±7.72

Fig. 4. Electrophoretic diagram of blood serum protein in *Rana nigromaculata nigromaculata*.



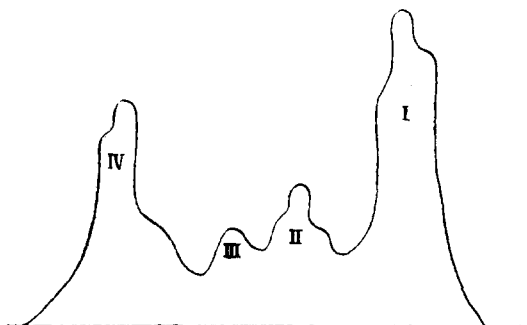
Fraction number	I	II	III	IV
Percentage composition	28.47 ±4.5	19.39 ±3.5	18.25 ±3.6	33.89 ±5

Fig. 2. Electrophoretic diagram of blood serum protein in *Cacopoides borealis*.



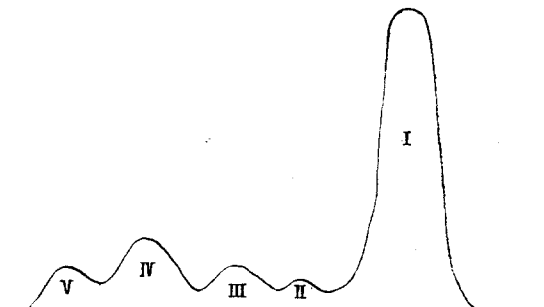
Fraction number	I	II
Percentage composition	45.42 ±6.5	54.58 ±5.4

Fig. 5. Electrophoretic diagram of blood serum protein in *Rana rugosa*.



Fraction number	I	II	III	IV
Percentage composition	47.38 ±1	16.88 ±1.4	13.08 ±2.4	22.76 ±1.8

Fig. 3. Electrophoretic diagram of blood serum protein in *Bufo bufo asiaticus*.



Protein name	Albumin	Alpha-I globulin	Alpha-II globulin	Beta-globulin	Gamma-globulin
Fraction number	I	II	III	IV	V
Percentage composition	58.94 ±1.2	7.41 ±1.9	9.23 ±1.9	1.3 ±0.57	11.42 ±1.3

Fig. 6. Electrophoretic diagram of blood serum protein in human.

At the end of each run, the filter paper strip was dried in a hot air oven at about 80°C to be stained for protein with bromophenol blue for about 30 minutes, washed four times in methylmercuric chloride solution for about five minutes and wet by paraffin oil to allow the light to go through it. The distribution of proteins was measured along the electrophoretic patterns by the Elphor H type photodensitometer. Diagrams were constructed from these measurement by plotting the optical density against the distanced migrated. The areas under these curves were measured by the OTT-Planimeter.

RESULTS

In general, the protein fractions in the blood serums did not separate as well as those of mammals, for some species of amphibian (eg. *Rana nigromaculata nigromaculata* and *Rana rugosa*). The electrophoretic mobility of blood serum protein was low and it was necessary to extent the length of time running to obtain adequate separation. In most samples, the number of fractions differed in the various species of amphibians.

1. *Bombina orientalis*: The protein pattern is separated into 5 distinct fractions as well as those of human. The protein fraction III is the highest, while the fraction I is the lowest among those of Anura reported. As a rule, the fraction I and II do not separate into very well. The electrophoretic mobility of this species is similar to that of human.

2. *Cacopoides borealis*: The most samples of blood serum protein taken from this species had 4 separated protein patterns. The fraction IV has not only the highest peak but also a high concentration. The fraction III is smaller than the fraction II but in general, the amount of components is similar each other. The fraction I is similar to that of *B. orientalis* in the Anura.

3. *Bufo bufo asiaticus*: There are 4 protein fractions of which the fraction I is the largest and composed about 47.38 percent of the total proteins. The fraction IV has lower concentration than the fraction I, but higher concentration than the fraction II and III. The fraction III composed the smallest concentration. The electrophoretic mobility is similar to that of *Bombina orientalis* and *Cacopoides borealis*.

4. *Rana nigromaculata nigromaculata*: Three protein reactions are present in the blood serum. The fraction III contains about 52.55 percent of the total protein. The electrophoretic mobility is very slow compared with those of above three species of Anura.

5. *Rana rugosa*: There are only two protein fractions in this species. The fraction I and II are relatively similar in amount of protein. The fraction II is generally higher than the fraction I in the peak as well as amount. The electrophoretic mobility of this species is similar to that of *Rana nigromaculata nigromaculata* and the concentration of the protein component is the lowest among the other species.

DISCUSSION

Moore (1948) reported that in the buffer system, 0.1 N sodium veronal —0.02 N diethyl barbituric acid, pH 8.6, the proportion of the various components of serum determined by the moving boundary method lie between the following values; albumin, 53 and 63%; α_1 -globulin, 4.0 and 8.0%; α_2 -globulin, 8.2 and 10.4%; β -globulin, 10.8 and 14.7%; γ -globulin, 8.5 and 14.7%. The result obtained by the present authors on the normal human blood serum to measure the accuracy of the present study was quite similar to the above values; albumin, 58.94%; α_1 -globulin, 7.41%; α_2 -globulin, 9.23%; β -globulin, 13%; γ -globulin, 11.42%. In general, the blood serum of mammalian is similar to the percentage composition of normal human blood serum protein.

But the component of albumin is the largest and globulin is slightly similar (Deutsch, 1945). It has, of course, been showed that there are characteristic individual electrophoretic patterns in birds as well as in mammals. The component of albumin, however, is the largest, and alpha, beta and gamma-globulin are severer than mammalian blood serum protein in the change. The result of investigation carried out on the blood serum protein of fish by Morris (1959) reported that albumin was 9 percent and globulin 91 percent in the Brown trout. In Rainbow trout, albumin was 49 percent and globulin 51 percent. The blood serum proteins of snake and fish was so remarkable in the number of

electrophoretic patterns that it was difficult to decide the names of proteins.

The fraction number of blood serum protein in *Bombina orientalis*, *Cacopoides borealis*, *Bufo bufo asiaticus*, *Rana nigromaculata nigromaculata* and *Rana rugosa* are very different and seems to depend upon the different species and family. The blood serum proteins in *Cacopoides borealis* and *Bufo bufo asiaticus* have separated into four electrophoretic patterns. As Morris (1959) has described that the names of proteins for each fraction have been decided by identifying the mobility of human blood serum protein, so the kind of fractions in amphibians are presumed as follow: fraction I protein may be albumin; fraction II protein, α -globulin; fraction III protein, β -globulin; fraction IV protein, γ -globulin. In *Rana nigromaculata nigromaculata* and *Rana rugosa* the fraction I protein may be considered to be albumin and the other fraction globulin according to Morris report that the protein fraction which localized at the initial albumin, its portion of the pattern has been globulin and the fastest migrating component albumin. If the fraction I protein shows generally remarkable change compared with globulin.

SUMMARY

Samples of blood serum obtained from 5 different species of amphibians have been analyzed for their protein by the methods of paper electrophoresis. The blood serum protein of *Bombina orientalis* as well as human separates into 5 protein fractions. The fraction III protein was the largest in its amount and the highest in its peak. The blood serum protein of *cacopoides borealis* and *Bufo bufo asiaticus* has separated into 4 protein fractions. In *Cacopoides borealis*, the fraction IV protein was the largest, while the fraction I was the largest in *Bufo bufo asiaticus*, *Rana nigromaculata nigromaculata* and *Rana rugosa* showed the differences in the number of these protein fractions but the peaks writing on the graph were similar one another. Albumin has been contained about an half of the total percentage composition. It suggests the more remote distance of these species is one another, the more evident the difference of these protein fractions will show in the percentage composition.

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