

Analysis of Serum Proteins of *Clarias batrachus* (Linnaeus) and *C. macrocephalus* (Gunther) by Electrophoresis

Amnuay Jondeung¹ and Uthairat Na Nakorn²

ABSTRACT

The serum proteins of Pla duk dan, *Clarias batrachus* and pla duk oui, *C. macrocephalus* have been analyzed by SDS-polyacrylamide gel electrophoresis. The electrophoretic patterns were found to be species-and sex-specific. Possible applications of specific electrophoretic markers are also discussed.

INTRODUCTION

The information about fish serum proteins have been reviewed by Boone (1964). Electrophoretic studies of plasma proteins of various species of fish have been reported by Sulya *et al.* (1961), Hattingh (1974) and Avtalion *et al.* (1975, 1976), who concluded that electrophoretic patterns of plasma proteins are specific at species level. On the basis of this specificity, several investigators have described the electrophoretic method for species determination in *Tilapia* by using serum proteins and different body tissues (Chen and Tsuyuki, 1970; Avtalion *et al.*, 1975, 1976; McAndrew and Majumdar, 1983) and in gray mullet by using myogen protein (Herzberg and Pasteur, 1975). Electrophoretic studies of serum proteins in infected fish revealed also the stage of disease, since Mulcahy (1969, 1971) found that there is a definite change in serum proteins in U.D.N. infected salmon and trout. He also described the electrophoretic technique for diagnosis of outbreaks of U.D.N.

There appear to have been no previous study on plasma proteins of local freshwater fish. The walking catfish of locally named "Pla duk" is an important cultured fish in Thailand. The information concerning the biochemistry of their plasma

proteins, in general, which might be used in fish management, breeding and pollution research, is not available. Therefore, the electrophoretic analysis of plasma proteins of two local catfishes, Pla duk dan, *Clarias batrachus* and Pla duk oui (*C. macrocephalus*) was undertaken and the electrophoretic patterns were found to be species - and sex - specific.

MATERIALS AND METHODS

Fish stocks:

The 15 samples of Pla duk dan *Clarias batrachus*, and Pla duk oui, *C. macrocephalus*, originated from commercial stock in Nonthaburi Province and were kept at Department of Aquaculture, Faculty of Fisheries.

Preparation of serum samples:

Soon after the fish were anaesthetized in 1:10,000 concentration of quinaldine, 0.5 ml of blood samples were withdrawn from caudal artery with 6 ml - syringe fitting with 1 - inch, 23 - gauge needle and containing 0.5 ml heparin solution (5,000 IU/ml). The blood samples were diluted with equal volume of saline and allowed to coagulate for 30 min. at room temperature. After centrifugation at 2200 rpm, all sera were stored at - 20 C° for electrophoresis.

1. Department of Genetics Faculty of Science Kasetsart Univ.

2. Department of Aquaculture Faculty of Fisheries Kasetsart Univ.

Electrophoresis:

Acrylamide solution, buffers and SDS - polyacrylamide gels (7 and 10%) at pH 8.9 were prepared as described by Ku-samran (1983). The electrophoresis was performed using buffer system Tris - glycine (pH 8.3.). Prior to electrophoresis, the sera samples were diluted with 1M Tris-glycerol sample buffer (1:1) containing 0.01% thymol blue as marker dye. The 2- mercaptoethanol was added to this sample in the concentration of 11% (V/V). Various amounts (5-20 ul) of diluted sera, as described above, were applied to SDS - polyacrylamide gels and electrophoresed for about 3 hr at 15 - 20 mA at room temperature. Parallel run with bovine serum albumin served as controls in each gel. The gels were stained at room temperature for 30 min in a solution of Coomassie Brilliant Blue R - 250 (0.2% w/v in water - methanol - acetic acid 46.6:46.5:7 v/v/v). Thereafter they were destained by elution in a solution of acetic acid - methanol - distilled water (70:50:1000 v/v/v).

RESULTS

Blood samples of mature *C. batrachus*, *C. macrocephalus* males and females were withdrawn from caudal artery and their sera were analyzed by using SDS - (7% and 10%) polyacrylamide slab gel electrophoresis. The results obtained in 7% - and 10% - polyacrylamide gels were different. The serum proteins were fractionated better in 10% - polyacrylamide gel than in 7% - ones, since many protein bands were separated in 10% - polyacrylamide. It might be that serum proteins of *Clarias* are mostly composed of small and fast moving components.

In 10% - polyacrylamide gel, the electrophoretic patterns of serum proteins were found to be relatively constant. Since a photographic picture (Fig. 1a) gives a less accurate record than the actual results, a schematic illustration of the different electropherograms in slab gel is given in Fig. 1b. The electrophoretic bands were enumerated according to their mobilities beginning by No.1

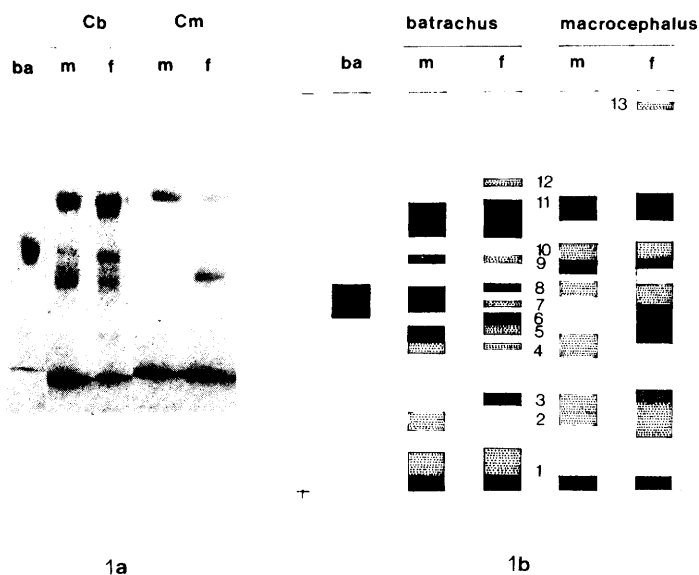


Fig. 1. Electropherogram of serum proteins (1a) and their schematic illustration (1b). The relative position of bands was determined on the basis of their mobilities in SDS-10% polyacrylamide gel. Band 1 was observed only in *C. batrachus*, while band 10 only in *C. macrocephalus*. Band 3 and 12, sexmarkers for females of *C. batrachus*. Band 3 and 13, sex-markers for females of *C. macrocephalus*. ba = bovine serum albumin; Cb = *C. batrachus*; Cm = *C. macrocephalus*; m = male; f = female

for the fast moving band and No. 13 for the slowest one.

The electrophoretic patterns of *Clarias* were found to be characteristic of each species. *C. batrachus* has a total of 11 bands whereas *C. macrocephalus* has 9. Two weak bands in position 2 and 3 and four dense bands in positions 6,8,9 and 11 appeared constant in both species. However, the distinction between the two species is clearly marked in position 1 and 10. Band 1 occurs only in electropherograms of *C. batrachus*, while band 10 only in ones of *C. macrocephalus*. The differences between two species were also observed in the middle region, from band 4-8, of electropherograms, but depended on sexes and will be discussed later.

The results also showed that the electropherograms of both species are different in males and females. These differences served as a key of sex - determination. The sex - identification of *C. batrachus* is based upon : (1) the presence in male of weak band 2 and in female of slightly dense band 3 and weak band 12 (2) the bands in positions 4-8 clearly separated in female, but not in male, only band 4,6 and 8 could be identified. In *C. macrocephalus*, the sex - identification is based upon : (1) the presence of dense band 3 and slightly dense band 13 in female (2) the bands in the positions 6 and 8 are seen in female, whereas only band 4 and 8 seen in male. From these differences, we conclude that there are at least a male - sex band and 2 female - sex bands in the electropherograms of *Clarias* species. The sex - marker bands found here seem to be a great deal more complex than that obtained in others fishes. Avtalion *et al.* (1975, 1976) observed the sex - markers only in male - electropherograms of *Tilapia aurea*, *T. vulcani* and in female - electropherograms of *T. nilotica*. This report presents firstly both male - and female - marker in *Clarias* species.

DISCUSSION

The electrophoretic patterns of sera proteins obtained here are specific at species level. These findings agree with previous observations on fish. The specific electrophoretic patterns for each species were reported in marine fishes (Sulya *et al.*, 1961), in *Tilapia* (Chen and Tsuyuki, 1970; Avtalion *et al.*, 1975, 1976) and in common mudfishes, *Lebeo capensis* and *L. umbratus* (Hatting, 1974). The patterns for each species are reproducible and difference with respect to the presence and absence of components (Booke, 1964). Therefore, the electrophoretic method has been used to identify *Tilapia* species (Chen and Tsuyuki, 1970; McAndrew and Majumdar 1983) and grey mullet species (Herzberg and Pasteur, 1974). The electrophoretic patterns found here could be used also to distinguish *C. batrachus* from *C. macrocephalus*.

The electropherograms were different in males and females of *Clarias* species. By using SDS - 10% polyacrylamide for analysis, a male - sex band 2 female - sex bands were identified. This finding is inconsistent with previous reports in *Tilapia*. Avtalion and Mires (1976) found that the electropherograms were the same in males and females of *Tilapia aurea*, except a male - sex band. Two male - sex and 2 female - sex bands were reported *T. vulcani* and *T. nilotica* respectively (Avtalion *et al.*, 1976). The sex - marker bands have been observed only in either male or female, but not in both sexes as seen in the *Clarias* species.

Interesting enough, the great variation was evident in the middle region of electropherogram, bands in positions 4 - 8. This region showed variation in both size and intensity of bands, except in *C. batrachus* females (Fig. 1). Their mobilities are almost the same as that of bovine albumin, which was run parallel as a control. The interpretations are following. (1) It seems likely that these protein components are polymorphism of albumin as described by Avtalion *et al.* (1976).

(2) Probably proteins of closely molecular weight were not well fractionated by the electrophoretic conditions used here. Therefore, different electrophoretic methods, e.g. disc - gel electrophoresis, isoelectrofocusing in a pH gradient as well as the different staining techniques could improve resolution.

Electrophoretic technique has a number of advantages for fish genetic research. Avtalion *et al.* (1975, 1976) used electrophoretic markers in *Tilapia* for selection of all - male hybrid brood, whereas McAndrew and Majumdar, (1983) used them for stock identification. Moreover, the electrophoretic markers could be used for advanced breeding scheme of fish as described by Moav *et al.* (1976). Before going to that point, the basic electrophoretic data of our local freshwater fishes are primarily needed to study.

ACKNOWLEDGEMENT

The authors wish to thank Asst. Prof. Dr. Yanyong Intararuksa, Faculty of Veterinary Medicine, for generously providing bovine serum albumin and Mrs. Christine Jondeung for her help in preparation of manuscript.

LITERATURE CITED

- Avtalion, R.R., Y. Pruginin and S. Rothbard. 1975. Determination of allogeneic and xenogeneic markers in the genus of *Tilapia*: I. Identification of sex and hybrid in *Tilapia* by electrophoretic analysis of serum proteins. *Bamidgeh* 27:8-13.
- Avtalion, R.R., M. Duczyminer, A. Wojdani and Y. Pruginin. 1976. Determination of allogeneic and xenogeneic markers in the genus of *Tilapia*. II. Identification of *T. aurea*, *T. vulcani* and *T. nilotica* by electrophoretic analysis of their serum proteins. *Aquaculture* 7:255-265.
- Avtalion, R.R. and D. Mires. 1976. Determination of allogeneic and xenogeneic markers in the genus of *Tilapia*. III. Electrophoretic identification of pseudohermaphroditism in *T. aurea* serum. *Aquaculture* 7:391-394.
- Booke, H.E. 1964. A review of variations found in fish serum proteins. *New York Fish and Game J.* 11(1) : 47-57.
- Chen, F.Y. and H. Tsuyuki. 1970. Zone electrophoretic studies on the proteins of *Tilapia mossambica* and *T. hornorum* and their F₁ hybrids, *T. zillii* and *T. melanopleura*. *J. Fish. Res. Bd. Canada* 27:2167-2177.
- Hattingh, J. 1974. The plasma proteins of *Lebeo umbratus* (Smith) and *Lebeo capensis* (Smith). *J. Fish. Biol.* 6:439-446.
- Herzberg, A. and R. Pasteur. 1975. The identification of grey mullet species by disc electrophoresis. *Aquaculture* 5:99-106.
- Ku-samran, T. 1983. Vertical slab SDS-PAGE. Workshop on Electrophoresis, Faculty of Science, Mahidol university, Bangkok (in Thai).
- McAndrew, B.J. and K.C. Majumdar. 1983. *Tilapia* stock identification using electrophoretic markers. *Aquaculture* 30:249-261.
- Moav, R., T. Brody, G. Wohlfarth and G. Hulata. 1976. Applications of electrophoretic genetic markers to fish breeding. I. Advantages and methods. *Aquaculture* 9 : 217-228.
- Mulcahy, M.F. 1969. Serum proteins changes in U.D.N.-infected Atlantic salmon: A possible method of diagnosis. *J. Fish. Biol.* 1:333-338.
1971. Serum protein changes associated with ulcerative dermal necrosis (UDN) in the trout *Salmo trutta* L. *J. Fish. Biol.* 3: 199-201.
- Sulya, L.L., B.E. Box and G. Gunter. 1961. Plasma proteins in the blood of fishes from the Gulf of Mexico. *Am.J. Physiol.* 200 (1) : 152-154.