Purification of Antihaemolytic Factor from the *Messerschmidia argentea.*

Yasuhiro TOMIHARA, Kazuhito OGIWARA*, Yasutetu ARAKI
Kuniki SHIMOJI, Keisuke FUKUMURA, Katumasa TOKUMURA
and Masatoshi NOZAKI.

Abstract. The antihaemolytic factor was purified from the *Messerschmidia argentea* by gel filtration. The purified antihaemolytic factor inhibited the haemolytic activity of *Chiropsalmus quadrigatus*. The antihaemolytic factor was stable to heating for 15 min at 100℃.

Key words. Box-jellyfish,antihaemolytic factor,*Messerschmidia argentea*

In some areas of Okinawa, Japan, the water extracts of the *Messerschmidia argentea* are used as first aid treatment of *Chiropsalmus quadrigatus* bites and for neutralization of fish poisoning. It is known that Box-jellyfish (*Chironex fleckeri*) venom contains the lethal, haemoletic and dermonecrotic properties (BAXTER and MARR, 1969) However, antihaemolytic factor have not been isolated as a homogenous preparation. In this paper we describe the purification of an antihaemolytic factor from the *Messerschmidia argentea.*

The leaves of *Messerschmidia argentea* (1,5kg) collected in Ikei island, was dissolved in water for 2 days. The extracts were filtered and the filtrate was lyophilized. Then, the dried powder was dissolved in water. After the insoluble material was removed by centrifugation, the supernatant was applied to a sephadex G-15 column (3.0 x 60cm) equilibrated with water. All Chromatography operations were performed at room temperature. 10 ml fractions was collected at a flow rate of 0.5ml per min, unless otherwise stated. Second fractions with antihaemolytic activity (indicated with a bar in Fig.1) were pooled. The active fraction was applied to a sephadex G-25 column (3.0 x 60cm) equilibrated with water. The active fractions indicated with a bar in Fig.2. The purified factor found to be a single by NMR and MS data. The purified factor of 1mg neutralized the 0.3 μ g of *Chiropsalmus quadriguatus* venom. The purified antihaemolytic factor was stable to heating for 15min at 100℃.

Chiropsalmus quadrigatus collected in Ishigaki island, was dissolved in water. Then, the extracts were filtered first through a cotton to remove the particles. After the insoluble material was removed by centrifugation, the supernatant was prepared to determine the haemolytic activity (SOUTHCOTT and KINGSTON, 1959). Antihaemolytic activity was deter mined by following method. *Chiropsalmus quadrigatus* venom (0.15ml) was mixed with 0.1ml of purified factor. After the mixture was left for 1hr at room temperature, 0.05ml of a 5 per cent suspension of washed red blood cells (horse or sheep) in saline was added. Antihaemolytic activity was determined by observing the degree of hemolysis.

*Department of Chemistry, College of Science, University of the Ryukyus

Stings by Box-jellyfish cause immediate skin pain, itching, swelling and skin blistering. Some of the stings also cause heart failure and difficulty in breathing. Victims stung by Box-jellyfish suffering from ugly scarring on the fingers or feet after sting. Box-jellyfish, *Chiropsalmus quadrigatus* bites are the main problems at the beaches of Okinawa, Japan. Box-jellyfish, *Chironex flekeri* antivenom has been shown to neutralize the venom of the Box-jellyfish, *Chiropsalmus quadrigatus* (BAXTER and MARR, 1974). The purified factor is to be expected for using as an adjunct of Antivenom for the treatment of Box-jellyfish bite (*Chiropsalmus quadrigatus*). Furthermore, the antihaemolytic factor may inhibit the haemolytic activity of other marine toxin. The structure of the antihaemolytic factor is currently under investigation.

ACKNOWLEDGEMENT

We are grateful to prof. Seiichi Yogi, Department of Chemistry, College of Science, University of the Ryukyus, for his helpful discussions.

REFERENCES

1. Baxter, E.H. and Marr,A.G.M.(1969) Sea Wasp (Chironex fleckeri) venom: Lethal, haemolitic and dermonecrotic properties. Toxicon, 7, 195.
2. Baxter, E. H. and Marr,A.G.M.(1974) Sea Wasp (Chironex fleckeri) antivenene: Neutralising potency against the venom of three other jellyfish species. Toxicon, 12, 233.
3. Southcott, R.V. and Kingston, C.W. (1959) Lethal jellyfish stings; A study in sea wasps. Med. J. Aust. 1,443.

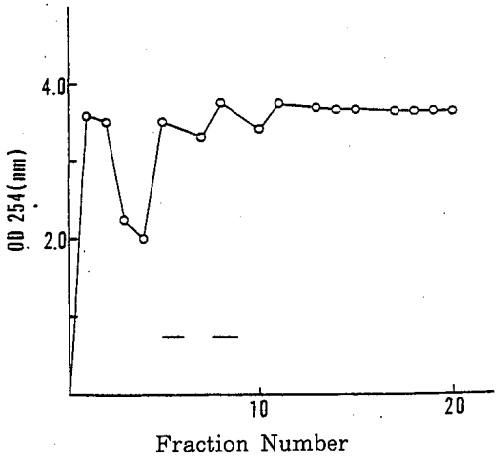

Fig. 1. Sephadex G-15 column chromatography of the water extracts of *Messerschmidia argentea*.

The water extracts of *Messerschmidia argentea* was applied to a Sephadex G-15 column (3.0×60cm) equilibrated with water. 10ml fractions were collected at a flow rate of 0.5ml per/min. The fractions containing antihaemolytic activity indicated with a bar.

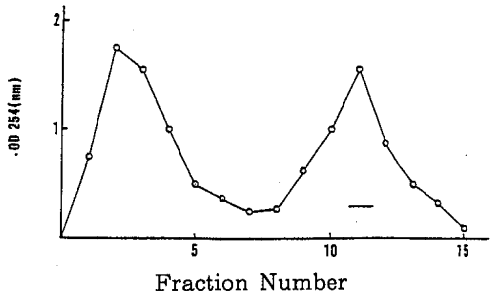

Fig.2. Sephadex G-25 columnchromatography of the water extracts of *Messerschmidia argentea*.

The active fractions from Sephadex G-25 were applied to a Sephadex G-25 column (3.0×60cm). The column was developed with water. 10ml fractions were collected at a flow rate of 0.5ml per/min. The active fractions indicated with a bar.

モンパノキの中の抗溶血因子の精製

富原靖博・萩原和仁*・新城安哲・下地邦輝
福村圭介・徳村勝昌・野崎真敏

＊琉球大学理学部化学科

モンパノキの水抽出物から Sephadex G－15 および Sephadex G－25のゲルろ過により、ハブクラゲ毒中の溶血作用を阻害する物質を単離した。その精製標品は、NMR や MS のデータから単一であることがわかった。

又、その標品は100℃, 15分間熱しても安定であった。