Biochemical studies on peptide alkaloids: Induction of ion selective mitochondrial swelling

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Summary. The effect of frangulanine, a cyclopeptide alkaloid isolated from *Hovenia dulcis* Thunb., on mitochondrial swelling was studied. Frangulanine induced mitochondrial swelling in 0.15 M KCl solution at the concentration of 6.5 μM. The alkaloid showed ion selectivity on the induction of mitochondrial swelling. Upon addition of frangulanine, mitochondria underwent swelling in 0.15 M KCl or RbCl solution but in neither NaCl nor LiCl solution.

Recently, a cyclopeptide alkaloid frangulanine has been isolated from *Hovenia dulcis* (Rhamnaceae)[1]. The conformation and crystal structure of the alkaloid has been reported previously[2,3] (figure 1). However its biochemical properties are yet unknown. The structure is very suggestive of an affinity with alkali metal ions. It was therefore expected that the peptide alkaloid may form a complex with alkali metal ions and act as an ionophore in biological or artificial model membranes. This communication deals with the affinity of frangulanine with alkali metal ions. In order to study the affinity of the alkaloid with alkali metal ions, induction of mitochondrial swelling by frangulanine was observed in 0.15 M RbCl, KCl, NaCl or LiCl solution. Isolated rat liver mitochondria undergo swelling with uptake of water and solutes when exposed to various kinds of uncoupling agents[4] and cyclopeptide antibiotics such as valinomycin[5,6].

Mitochondria have been shown to undergo ion-selective swelling upon addition of peptide antibiotics such as valinomycin and gramicidin A[5-7]. Frangulanine showed ion selectivity on the induction of mitochondrial swelling.

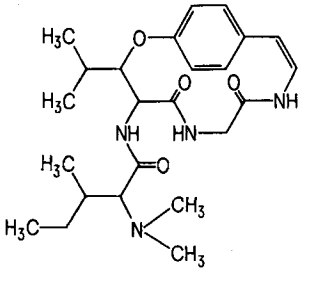

Fig. 1. Chemical formula of frangulanine.

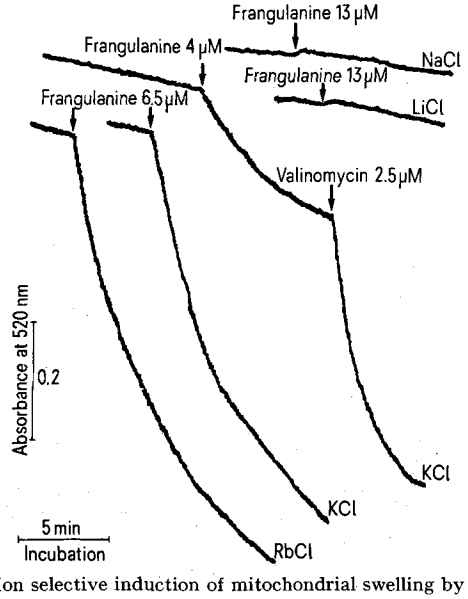

Fig. 2. Ion selective induction of mitochondrial swelling by frangulanine.

Materials and methods. Frangulanine was isolated from dried and crushed root bark of *Hovenia dulcis* Thunb., by the method as reported previously[1], and was used as a solution of dimethylformamide. Tris-(hydroxymethyl)-aminomethane and valinomycin were purchased from Sigma Pharmaceutical Co. and Boehringer, Mannheim, respectively. Other reagents were of the purest grade commercially available.

Rat liver mitochondria were prepared by the method of Schneider[8] by using a medium containing 0.25 M sucrose, 0.5 mM EDTA, and 20 mM Tris-HCl, pH 7.4. Mitochondrial swelling was observed by tracing the decrease of absorbance at 520 nm by using Hitachi Recording Spectrophotometer EPS-3T. Mitochondrial protein was assayed by the method of Lowry et al.[9].

Results and discussion. The effect of frangulanine on mitochondrial swelling was studied in 0.15 M LiCl, NaCl, KCl or RbCl solution, buffered in each case by 20 mM Tris-HCl, pH 7.4. As shown in figure 2, in KCl or RbCl solution, frangulanine at 6.5 μM caused marked decrease of the absorbance at 520 nm, showing the induction of the mitochondrial swelling. The swelling induced at lower concentration of frangulanine (4.0 μM) was accelerated by addition of valinomycin (2.5 μM). In contrast, mitochondria in 0.15 M NaCl or LiCl solution were shown not to swell by the addition of the alkaloid, even at the higher concentration (13 μM). These results apparently indicate that frangulanine shows ion selectivity on the induction of mitochondrial swelling.

This ion selectivity of the alkaloid might be caused by the formation of a complex with K^+ or Rb^+ and act as an ionophore in the mitochondrial inner membranes as does valinomycin. It would therefore be of great interest to investigate whether the formation of a complex with K^+ is closely related with the biological significance of the alkaloid in plants. Since such alkaloid compound has been known to be chiefly localized in the root bark of *Hovenia dulcis*, it is possible that the alkaloid may be involved in the absorption of nutrients from the soils, especially alkali metal ions.

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