IONOPHORETIC ACTIVITIES OF OLIGOPEPTIDE LACTONES AND RESIN-GLYCOSIDES IN HUMAN ERYTHROCYTES

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Oligopeptide lactones (theonellapeptolides) isolated from the Okinawan marine sponge Theonella swinhoei and resin-glycosides (merremosides) from the tuber of an Indonesian medicinal plant Merremia mammosa were examined regarding their activities in transporting Na^+, K^+, and Ca^{++} ions into human erythrocytes. Each of these lactones, which had been shown, using a supported liquid membrane, to have an ionophoretic effect on the alkali metal ions, transported the ions to a different extent. The ion transporting activities of these compounds were completely lost when the macrocyclic lactone structures were cleaved by sodium methylate. Resin-glycosides with an additional branched glycosyl residue showed much greater ion transporting activities than those without it.

KEYWORDS human erythrocyte; ion transporting activity; ionophore; theonellapeptolide; merremoside; oligopeptide lactone ionophore; resin-glycoside ionophore

Ionophores, the organic moieties capable of forming liquid-soluble complexes with cations, transport ions across hydrophobic barriers, including artificial and biological membranes. They have been extensively used in the studies of biology[1] and analytical chemistry.[2] We recently developed a new apparatus provided with a supported liquid membrane for the measurement of ion transporting activity and found new ionophoretic natural products: an oligopeptide lactone theonellapeptolide Id and two resin-glycosides merremosides a and h_1.[3] In this paper, we report the ion transportability across human erythrocyte membranes of 5 oligopeptide lactones isolated from the Okinawan marine sponge Theonella swinhoei[4] and 8 resin-glycosides from the tuber of an Indonesian medicinal plant Merremia mammosa.[5]

MATERIALS AND METHODS

Human blood from healthy donors was collected in heparin solution immediately before use. The blood plasma and buffy coat were removed by centrifugation and the erythrocytes were washed with isotonic N-2-hydroxyethylpiperazine-N'-2-ethanesulfonate (HEPES) buffer 3 times at room temperature. The basal composition of the isotonic HEPES buffer was (mM): $CaCl_2$ (1), $MgCl_2$ (1), glucose (5) and HEPES (10), pH 7.0. To this buffer we added: NaCl (1), KCl (2) and choline chloride (136) for the measurement of Na^+ transport, NaCl (136), KCl (1) and choline chloride (2) for K^+ transport, and NaCl (137) and KCl (2) for Ca^{++} transport. The washed cells were resuspended in the corresponding medium to give a final cell concentration of 1 x 10^9/ml. The cell suspension was incubated at 37°C and 5 μCi of metal ions were added and incubated for another 12 minutes. Then, the experiment was started by adding an ionophore solution. At intervals, 0.2-ml samples were drawn, layered on di-n-butylphthalate solution (d_{20} 1.04), and centrifuged (15,000 rpm, 1 min) to separate the erythrocytes from the medium. After removing the medium and the phthalate solution, the pelleted cells were hemolyzed by adding 0.2 ml of a hypotonic buffer (1.89 mM Na_2HPO_4- 3.11 mM NaH_2PO_4, pH 7.0). One-tenth ml of 10% trichloroacetic acid was then added and the hemolysate was centrifuged at 15,000 rpm for 5 min. A sample of the supernatant was used for counting the radioactivity in a liquid scintilation spectrometer (Aloca, LSC-950).

RESULTS AND DISCUSSION

Metal Ion Transport by Theonellapeptolides

The transport of alkali metal ions into erythrocytes in the presence of the oligopeptide lactones

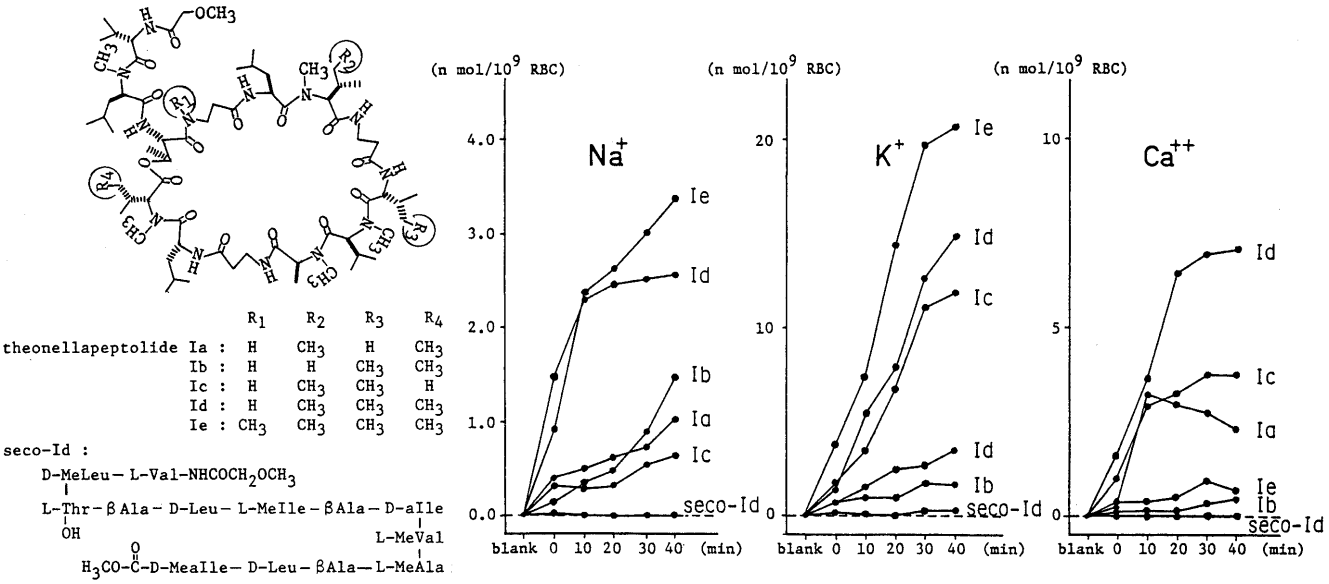

Fig. 1. Metal Ion Transport by Theonellapeptolides (0.01 μmol/10^9RBC)

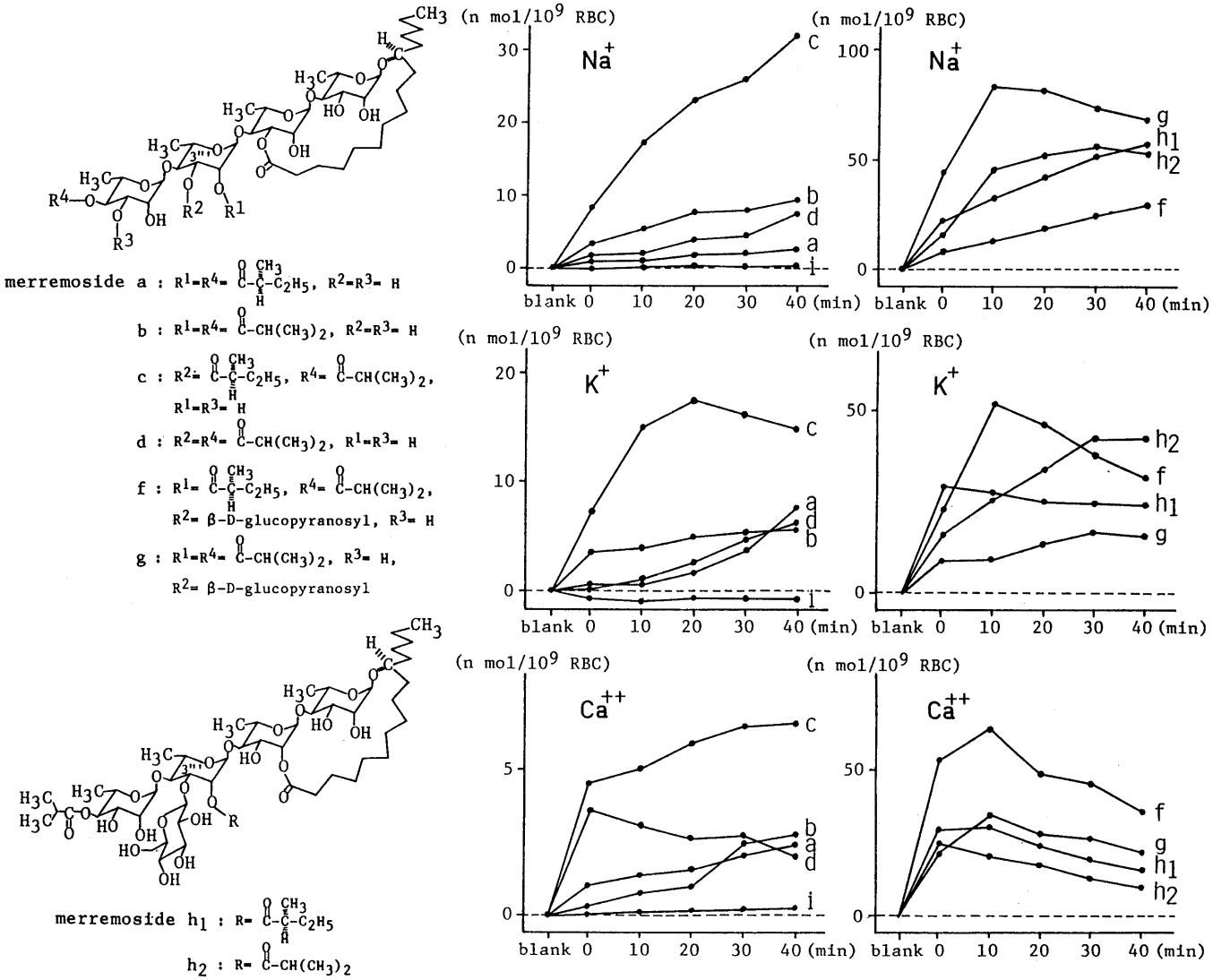

Fig. 2. Metal Ion Transport by Merremosides (0.10 μmol/10^9RBC)

(theonellapeptolides),[4] one of which (theonellapeptolide Id) had been shown to have an ionophoretic activity using a supported liquid membrane,[3] was examined (Fig. 1). Five kinds of these tridecapeptides differing in their amino acid composition demonstrated ion transporting activity across erythrocyte membranes, to a different extent. Theonellapeptolide Id showed the highest activity among them; Ie transported Na^+ and preferentially K^+. The compound named seco-Id, which was obtained from Id by treatment with $NaOCH_3$-CH_3OH, had no activity, indicating that the macrocyclic lactone structure was essential to exert the ion transporting activity of these peptides.

Metal Ion Transport by Merremosides

In connection with the ionophoretic activities of merremosides a and h_1 previously shown by using a supported liquid membrane,[3] ion transportability across erythrocyte membranes of merremosides[5] was tested (Fig. 2). Among these glycosides, f, g, h_1 and h_2 that have an additional glycosyl residue branching at the 3"' position showed much greater activity than those without such branching (a, b, c, d). The highest activity was obtained with merremoside g in transporting Na^+, with f, h_1 and h_2 in transporting K^+, and with f in transporting Ca^{++}. As with the theonellapeptolides, cleavage of the macrocyclic lactone structure (forming merremoside i[5b]) by treatment with $NaOCH_3$-CH_3OH eliminated the activity completely, again suggesting the macrocyclic structure of the molecules to be essential.

Judging from the structure of merremosides, one cannot assume that they have large enough cavities in their lactone rings to trap the metal ions. Instead, a molecular conformation that may be formed by the presence of the lactone rings appears to provide an ion-trapping activity.

Metal ion transport across erythrocyte membranes does not always appear to be commensurate with that across a liquid membrane. Theonellapeptolide Id transported ions in the order of $K^+ > Na^+ > Ca^{++}$ across a liquid membrane,[3] but into erythrocytes it was in the order of $K^+ > Ca^{++} > Na^+$. The activities of merremosides a and h_1 measured by a liquid membrane system were in the order of $Ca^{++} > Na^+ > K^+$ and $Na^+ > K^+ \gg Ca^{++}$, respectively. These were not in parallel with the results with erythrocytes. When examined using human erythrocytes, merremoside h_1, with a branched glycosyl residue at the 3"' position, had much greater Ca^{++}-transporting activity than merremoside a which lacks the glycosyl residue at the 3"' position. The distinct difference between ion transport across the artificial and biological membranes may result from the more complex architecture of biological membranes over simple hydrophobic barriers. The branched glycosyl residue at the 3"' position in the merremosides may modify the ion transport across biological membranes.

Resin-glycosides such as merremosides have been demonstrated in the present paper for the first time to transport ions into human erythrocytes. These compounds are likely to form through lactone rings a certain conformation capable of trapping metal ions in their sugar moieties. The large number of ionophores so far reported have a hydrophilic macrocyclic structure in their molecules. As reported in this paper, however, compounds that lack a sufficient number of heteroatoms to trap a metal ion in the cyclic structure can also exert ion transporting activity.

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