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CHANGES IN STRUCTURAL ORGANIZATION OF SURFACE MEMBRANE DURING ERYTHROCYTE MATURATION

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Summary

The effect of concanavalin A and its succinylated derivative on cell agglutination and potassium compartmentation of mature and immature erythrocytes was observed. The binding of tetravalent concanavalin A to the surface glycoproteins of rabbit erythrocytes leads to a change in the properties of the surface membrane, which results in an induction of cell agglutination and concomitant release of potassium from the cells. Both of the phenomena induced by concanavalin A are temperature dependent, and observed at above 15°C.

Divalent succinylated concanavalin A, lacking the inducing activity of surface glycoprotein cross-linking into patches and caps, caused neither cell agglutination nor change in the potassium compartmentation of erythrocytes and reticulocytes.

In the case of immature reticulocytes, however, remarkable agglutination of the cells was induced without a change in the potassium compartmentation after treatment with tetravalent concanavalin A.

It is suggested that changes in the molecular organization of the surface membrane occur in which potassium compartmentation of the reticulocytes becomes more susceptible to surface glycoprotein cross-linking during cellular maturation.

Introduction

Carbohydrate binding protein concanavalin A, which binds to mannose-like sites on the cell surface [1], has become a useful probe for the study of the structural organization of the surface membrane [2,3], and the mechanism of cell recognition [4,5] or cellular metabolic regulation [6]. The difference

between the surface membranes of normal and transformed [7,8], or mature and immature cells detected by concanavalin A is a subject of widespread interest [9,10]. Inoue et al. [11] reported that the agglutinability of rabbit reticulocytes decreased with the cellular maturation without loosing the number of concanavalin A binding sites per cell. Concanavalin A-induced agglutination of reticulocytes and erythrocytes showed differing temperature dependency (the former was observed at a lower temperature than the latter) and it was suggested that the membrane fluidity and/or the topographical distribution of concanavalin A receptor sites on these cell surfaces changed during erythrocytes maturation. [11].

It is necessary to know what kind of intracellular changes occur after the interaction of surface receptors of these two cell types with concanavalin A for the study of the functional organization of the glycoproteins on the cell surface. With this concept in mind, we report here the effect of concanavalin A and its succinylated derivative on cell agglutination and the potassium compartmentation of mature and immature erythrocytes.

Materials and Methods

Materials. Concanavalin A was purified from Jack bean meal by the method of Agrawal and Goldstein [12]. The divalent succinylated derivative of concanavalin A, having the same affinity for α-methyl-D-glucoside as native tetravalent concanavalin A, was prepared by the method of Gunther et al. [13], and further purified by affinity chromatography with Sephadex G-100. The lectins thus obtained were dissolved in 10 mM Tris · HCl buffer (pH 7.4) containing 0.34 M mannitol, 1 mM $CaCl_2$ and 1 mM $MnCl_2$. All reagents used were of reagent grade.

Cells. Reticulocytes were obtained from peripheral blood of phenylhydrazine-injected rabbits by the method of Borsook et al. [14]. The rabbit erythrocytes and reticulocytes were washed three times with 10 mM Tris · HCl (pH 7.4) containing 0.34 M mannitol and used for the experiments in the same medium.

Cell agglutination. Cell agglutination was tested in the medium used for cell washing for 30 min at varying temperatures, and scored from — to ++++ as described previously [15]. Cell concentration used for all experiments was $8 \cdot 10^6$ per ml.

Measurement of potassium release. Suspended cells ($8 \cdot 10^6$/ml) were incubated in the same medium used for cell agglutination at varying temperatures, and changes in potassium concentration of incubation mixture were measured continuously by K^+-sensitive electrode (Electronic Instrument Ltd., Model C-KN33C) connected to a pH meter (Toa Electronic Ltd., Model HM-20B). Potassium content remaining within the cells was measured by releasing it with 0.1% Triton X-100 after 30 min incubation at 30°C unless otherwise stated.

Viability test of cells. The viability of the cells was tested by three methods, phase contrast microscopy, dye exclusion test of trypan blue and measurement of hemoglobin released from the cells at the end of each incubation. Erythrocytes treated with 300 μg/ml concanavalin A at 37°C for 30 min was resuspended in the incubation medium containing 20 mM α-methyl-D-glucoside

and incubated for 5 min at 25°C, and they were observed under phase contrast microscopy. Most cells were dispersed into single cells, and a small amount of microaggregates, mainly composed of 3—20 cells, were observed. However, a negligible amount of ghosts was found before or after incubation (3/1000 cells counted).

The erythrocytes thus treated with α-methyl-D-glucoside were added to trypan blue dissolved in the incubation medium in a final concentration of 0.05% and further incubated for 5 min at 25°C. The number of stained cells was less than 1.2% at the end of incubation.

The extent of hemoglobin released from the incubating cells was also measured at the end of incubation to cover the uncertainty of the percentage of stained cells due to microaggregates. At the end of the incubation, they were centrifuged at 3000 rev./min for 5 min at 4°C. The extent of hemoglobin in the supernatant solution was measured spectroscopically at 410 nm, and found to be 2.1% of the total hemoglobin released by 0.1% Triton X-100 in the case of concanavalin A treatment, while that of the control was 1.6%.

Treatment of reticulocytes with concanavalin A under the same conditions showed a similar degree of change in viability. In the case of succinylated concanavalin A treatment, a similar degree of change in viability was also observed. No differential effect of lectin cytotoxicity was observed between the two cell types under these criteria at any conditions examined.

Results

Changes in potassium compartmentation of mature erythrocytes

Fig. 1 shows the effect of concanavalin A on the potassium compartmentation of rabbit erythrocytes. There is a rather rapid induction of K^+-release from the cells after an addition of concanavalin A (Curve 1). Concanavalin A-induced K^+-release from the cells is specifically inhibited by α-methyl-D-glucoside, a haptenic inhibitor of this lectin (Curve 3). The addition of sugar to the reaction mixture during incubation also inhibited K^+-release from the cells suggesting the reversible nature of concanavalin A action (Curve 2). Addition of Triton X-100 (0.1%) to the incubation mixture caused a sudden release of K^+ remaining in the cells. Concanavalin A-treated cells seemed to be more resistant to Triton X-100 induced hemolysis than the control, probably due to cell agglutination.

Effect of valency of concanavalin A on cell agglutination and potassium compartmentation of erythrocytes

Fig. 2 shows the effect of concanavalin A (tetravalent) and succinyl concanavalin A (divalent) on the cell agglutination and K^+-release from mature erythrocytes. The extent of K^+ released from the cells increased as a function of concanavalin A concentration. The degree of cell agglutination also showed similar dependency on the concentration of the lectin. On the other hand, divalent succinyl concanavalin A caused neither cell agglutination nor change in potassium compartmentation of erythrocytes under the same conditions as tetravalent concanavalin A. This data suggests that there is a close relationship

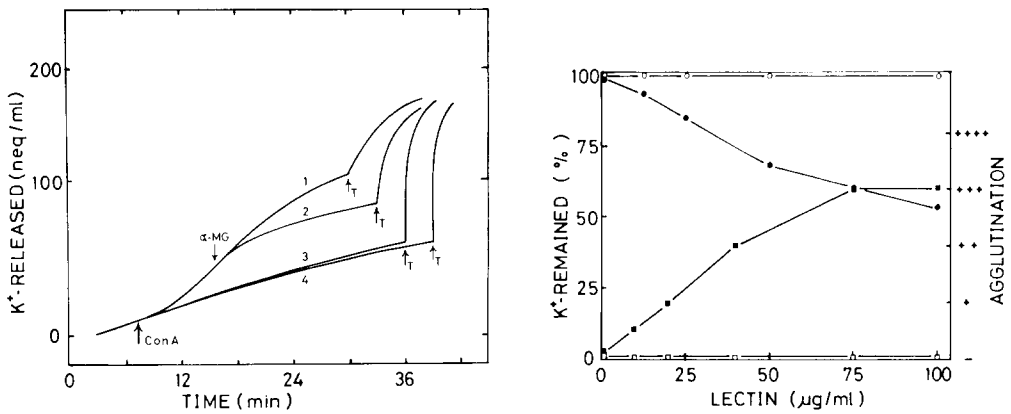

Fig. 1. Effect of concanavalin A on the potassium compartmentation of rabbit erythrocytes. Rabbit erythrocytes ($1.8 \cdot 10^9$/ml) were suspended in 5 ml of medium described in the text, and treated with native concanavalin A (300 μg/ml) at 37°C in the presence (Curve 3) or absence (Curve 1) of 10 mM α-methyl-D-glucoside (α-MG). In curve 2, 10 mM of sugar was added to the incubation mixture 8 min after addition of concanavalin A. At the end of each incubation, all K^+ remaining within the cells was released by the addtion of 0.1% Triton X-100 (T). Other conditions are described in the text. Curve 4, control.

Fig. 2. Effect of concanavalin A and its succinylated derivative on the potassium compartmentation and cell agglutination of mature erythrocytes. Erythrocytes ($8 \cdot 10^6$/ml) were incubated in the same medium as Fig. 1 with concanavalin A or succinyl concanavalin A at 30°C for 30 min. The extent of K^+ remaining within the cells was calculated after releasing it from the cells by Triton X-100. Agglutination induced by concanavalin A (■———■), or by succinyl concanavalin (□———□); K^+ remaining within the cells after treatment with concanavalin A (●———●), or with succinyl concanavalin A (○———○).

between the two phenomena induced by the lectins and their valence and/or the degree of glycoprotein cross-linking on the cell surface.

Temperature dependent response of erythrocytes

Fig. 3 shows the effect of temperature on the concanavalin A-induced cell agglutination and the change in potassium compartmentation of mature erythrocytes. Both of these phenomena induced by concanavalin A were temperature dependent, and were observed at temperatures above 15°C. However, succinyl concanavalin A lacks such activity at any temperature examined. This indicates that cell agglutination and the change in potassium compartmentation follow a common pattern as a function of lectin concentration, incubation temperature, and valency of concanavalin A.

Effect of concanavalin A on cell agglutination and potassium compartmentation of reticulocytes

Fig. 4 shows the effect of concanavalin A and the succinylated derivative on the cell agglutination and potassium compartmentation of reticulocytes. In the case of reticulocytes, there was no such relationship between the two phenomena induced by concanavalin A as observed with mature erythrocytes. Reticulocytes treated with concanavalin A showed remarkable agglutination without a modification in potassium compartmentation. Neither cell agglutination nor changes in potassium compartmentation were seen when reticulocytes were treated with succinyl concanavalin A.

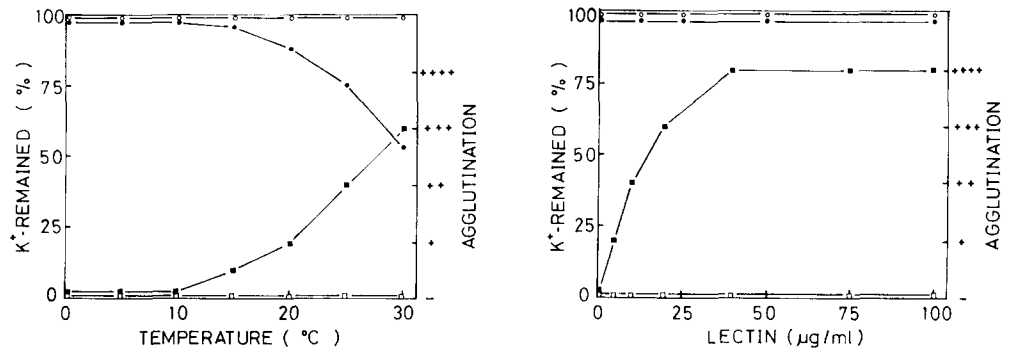

Fig. 3. Effect of temperature on the cell agglutination and potassium compartmentation of mature erythrocytes. The cells were incubated with lectins for 30 min at varying temperatures. The concentration of lectins used for incubation was 100 μg/ml. Other conditions were the same as Fig. 2. Agglutination induced by concanavalin A (■———■), or succinyl concanavalin A (□———□); K^+ remaining within the cells after incubation with concanavalin A (●———●), or with succinyl concanavalin A (○———○).

Fig. 4. Effect of concanavalin A and succinyl concanavalin A on cell agglutination and potassium compartmentation of reticulocytes. All conditions were the same as Fig. 2. Reticulocytes agglutination induced by concanavalin A (■———■), or by succinyl concanavalin A (□———□); K^+ remaining within the cells after incubation with concanavalin A (●———●), or with succinyl concanavalin A (○———○).

Temperature dependent response of reticulocytes

Fig. 5 shows the effect of temperature on the cell agglutination and K^+-release from reticulocytes. Like the mature erythrocytes, concanavalin A-induced agglutination of reticulocytes was also temperature dependent. However, no change in potassium compartmentation was induced by the lectin. Again, succinyl concanavalin A caused neither cell agglutination nor K^+-release from reticulocytes. In the case of reticulocytes, there was no parallel relationship between the two phenomena induced by concanavalin A under any conditions examined.

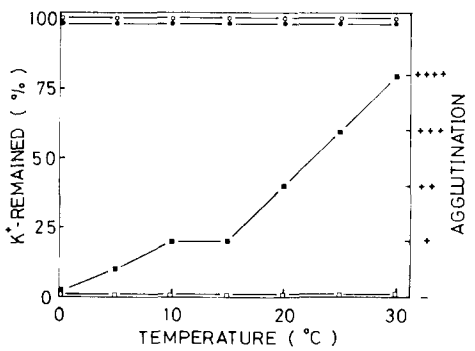

Fig. 5. Effect of temperature on the cell agglutination and potassium compartmentation of rabbit reticulocytes. Conditions were the same as Fig. 3. Reticulocyte agglutination by concanavalin A (■———■), or by succinyl concanavalin A (□———□); K^+ remaining within the cells after treatment with concanavalin A (●———●), or with succinyl concanavalin A (○———○).

Discussion

The data show that the binding of tetrameric concanavalin A to the surface glycoproteins of rabbit erythrocytes leads to a change in the properties of the surface membrane, which results in a concomitant induction of cell agglutination and a change in the potassium compartmentation of the erythrocytes. Both of the phenomena induced by concanavalin A were temperature dependent, and observed above 15°C. The dimeric succinyl concanavalin A induced neither cell agglutination nor K^+-release of erythrocytes under any conditions tested. Similar phenomena were observed with Ehrlich ascites tumour cells [16,17]. Namely, tetravalent concanavalin A, but not divalent succinyl concanavalin A, caused significant cell agglutination with concomitant changes in potassium compartmentation of the ascites cells. Both of these events were inhibited by α-methyl-D-glucoside or by lowering the incubation temperature under 10°C. Gunther et al. [13] suggested that tetrameric concanavalin A, but not succinyl concanavalin A, induced cross-linking of the surface receptors into micropatches and eventually caps on certain cells, a process inhibited by α-methyl-D-glucoside or a low temperature (10°C). It seems likely that the same mechanism of concanavalin A action that underlies glycoprotein cross-linking on cell surface is also operating in the case of the change in the potassium compartmentation of mature erythrocytes.

In contrast, a remarkable agglutination was observed with no induction of K^+-release from cells after the addition of tetravalent concanavalin A in the case of reticulocytes. Therefore glycoprotein cross-linking by itself is not sufficient to bring about a change in the potassium compartmentation of reticulocytes. Such a difference in the induction of K^+-release from the two cell types may be due to the difference in the number of surface receptors per cell for this lectin. However, the number of concanavalin A binding remained unchanged during erythrocyte maturation [11]. This suggests that the membrane changes other than the number of lectin binding are likely to account for the difference in the induction of K^+-release from the two cell types. The reason why the glycoprotein receptor cross-linking of the erythrocyte surface membrane induced by concanavalin A leads to the change in potassium compartmentation, while that of reticulocytes does not, remains unclear.

Ji et al. [18] reported that perturbation of components on the outer membrane surface could be translated to the cell interior by transmembrane control mechanism. If the induction of K^+-release depends on such mechanism, the differences observed between the two cell types may be caused by structural changes in the surface receptor moiety. Balduini et al. [19] suggested that the glycoproteins of the surface membranes undergo significant chemical modifications with erythrocyte maturation and aging, mainly consisting of a decrease in sialic acid and galactosamine, which might cause erythrocyte sequestration by reticuloendothelial system and consequently could induce lysis of the cells. From these data, it is suggested that the surface membrane changes the structural organization in which the potassium compartmentation of immature erythrocytes becomes more susceptible to glycoprotein cross-linking during erythrocyte maturation and/or aging.

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