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INTERACTION OF SOLANUM TUBEROSUM AGGLUTININ WITH HUMAN PLATELETS

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SUMMARY: Solanum tuberosum agglutinin (STA) binds to the surface of human platelets and leads to their agglutination. Lectin staining shows that ^{125}I-STA most intensely labels a major platelet membrane glycoprotein identified as GPIIIa followed by GPIV. STA does not induce release reaction, TXB_2 formation or platelet protein phosphorylation. Since STA-induced agglutination is independent of intracellular metabolism of platelets, STA may prove to be a useful tool to explore the clinical condition in which the composition of platelet membrane protein is altered.

INTRODUCTION: Lectin binds sugar on the cell membrane and induces cell agglutination by cross-linking of the adjacent cells (1,2). In platelets, some lectins induce not only agglutination but platelet activation such as the release reaction (3-6) or platelet protein phosphorylation (6). However, there are no reports concerning about demonstrable lectins which induce agglutination without any activation of platelets. Blood group-nonspecific potato lectin, solanum tuberosum agglutinin (STA), was first described by Marcusson-Begun (7), and the studies of its purification and characterization have been reported (8,9). In this study, we presented data which showed that STA induced platelet agglutination without any activation. Since platelet aggregating agents such as ADP, collagen and arachidonic acid are influenced by the change of intracellular metabolism or drug intakes, STA may be used as a simple probe to explore the clinical condition in which the composition of platelet surface glycoprotein is altered.

Abbreviations: GPIIIa, glycoprotein IIIa; GPIV, glycoprotein IV; TMB-8, 8-(N,N-diethylamino)-octyl-3,4,5-trimethoxybenzoate; PGE_1, prostaglandin E_1

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MATERIALS AND METHODS

Blood was collected from volunteers in plastic syringes utilizing 3.8% w/v sodium citrate as the anticoagulant (9:1). Washed platelets were prepared by the method of Mustard et al. (10) except that the final platelet pellet was resuspended in Ca^2-free Tyrode's buffer with glucose (1 mg/mℓ) to achieve a final count of $30 \times 10^4/\mu\ell$. Platelet aggregation and ATP release was measured in the lumiaggregometer (Chrono-Log Co.). STA, wheat germ agglutinin (WGA), concanavalin A (Con A), N-acetyl-D-glucosamine (GlcNAc), Di-N-acetylchitobiose [$(GlcNAc)_2$] and Tri-N-acetylchitotoriose [$(GlcNAc)_3$] were obtained from E.Y. Laboratories Inc., San Mateo, CA. The purity of lectins was determined by SDS gel electrophoresis. Iodoacetate and antimycin A were obtained from Sigma Chemical Co., St. Louis, Ma. TMB-8 was made by the method of Malagoni et al. (11). PGE_1 was gift from Ono Pharmaceutical Co., Osaka, Japan. $Na^{125}I$ (350 mCi/mℓ) and carrier-free ^{32}Pi were obtained from New England Nuclear (NEN), Boston, Mass. The ^{125}I-labelling of STA was performed by chrolamin-T method of Hunter (12). The membrane components which bound to lectin were identified by use of ^{125}I-lectin staining method described by McGregor et al. (13) with minor modification. Washed platelets ($150 \times 10^4/\mu\ell$) was solubilized in the solubilizing buffer [62.5 mM Tris-HCl, 2.3% SDS, 10% glycerol (w/v), pH 6.8] with 5% 2-mercaptoethanol. After separation of solubilized platelet protein by SDS-polyacrylamide gel electrophoresis (12.5%) under the conditions specified by Laemli (14), the gel was stained with ^{125}I-STA in the presence of 0.2% BSA to prevent nonspecific binding. The dried gel was exposed to Fuji X-ray film at -70°C for 1-2 wk. Luciferin-luciferase (Sigma) was used in the study of ATP release according to the method of Feinman (15). The release of serotonin was measured with platelets preloaded with [^{14}C] serotonin (60 mCi/mmol, Amersham Corp., Arlington Heights, Ill) according to the method of Haslam et al. (16). Radioimmunoassay was performed using commercially available kits for β-thromboglobulin (β-TG, Amersham), platelet factor 4 (PF4, Abbot Laboratories, North Chicago, Ill) and thromboxane B_2 (TXB_2, NEN). Labelling of platelets with ^{32}Pi was carried out as described by Lyons et al. (17).

RESULTS AND DISCUSSION

In this paper, we have demonstrated that STA, one of the GlcNAc specific lectins (18), induces platelet agglutination in a dose dependent manner (Fig. 1A), and that, by lectin staining, ^{125}I-STA most intensely labels a major platelet glycoprotein of 114,000 daltons followed by 97,000 daltons, which were identified as GPIIIa and GPIV, respectively, according to the study of Phillips (19) (Fig. 2). Similar pattern of staining was reported in the case of Con A (glucose, mannose determinant) (4). STA (100 μg/mℓ)-induced platelet agglutination was not inhibited by 50 mM GlcNAc (Table 1), but almost completely inhibited by 0.3 mM [$(GlcNAc)_3$] (Fig. 1B). [$(GlcNAc)_2$] was a less potent STA inhibitor than [$(GlcNAc)_3$] (Table 1). These data suggest that STA binds to the oligosaccharides which contain β-linked GlcNAc residues on platelet membrane (mainly GPIIIa and GPIV) and leads to platelet agglutination.

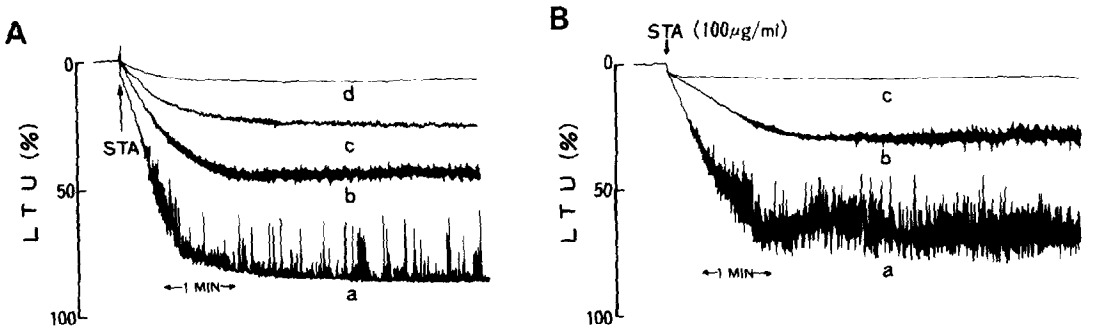

Fig. 1. (A) STA-induced platelet agglutination. Platelet count is $30 \times 10^4/\mu\ell$. The concentration of STA is (a) 100 μg/mℓ; (b) 60 μg/mℓ; (c) 40 μg/mℓ; (d) 10 μg/mℓ. The change of light transmission after 5 min induced by STA (100 μg/mℓ), expressed as percentage (mean±SD) of that by WGA (100 μg/mℓ), was 113±9% (mean±SD, n=5). (B) Effect of $[(GlcNAc)_3]$ on STA-induced platelet agglutination. 490 μℓ of washed platelets were preincubated at 37°C with 10 μℓ of $[(GlcNAc)_3]$ solution or saline (for control) for 5 min, prior to STA (100 μg/mℓ in final). (a) control; (b) 0.15 mM $[(GlcNAc)_3]$; (c) 0.3 mM $[(GlcNAc)_3]$.

STA-induced platelet agglutination also occurred in formalin-fixed washed platelets (data not shown) and in washed platelets preincubated with metabolic inhibitors such as iodoacetate (0.5 mM) plus antimycin A (2 μM) (Table 1). Ca^{2+} chelator; EDTA (5 mM), aspirin (100 μg/mℓ), PGE_1 (2 μM), Ca^{2+} blocker; diltiazem (500 μM) and TMB-8 (500 μM) and calmodulin inhibi-

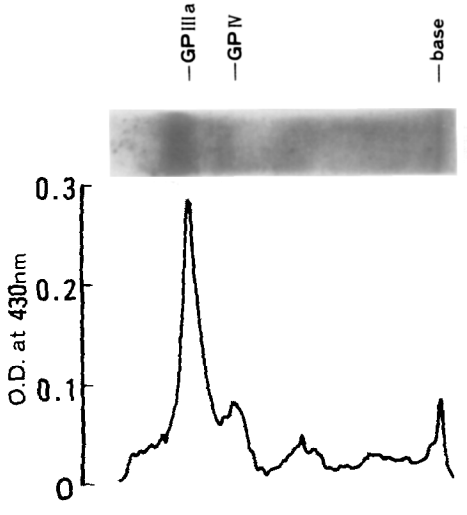

Fig. 2. Lectin (^{125}I-STA) staining of whole platelets. Glycoproteins of whole platelets solubilized in SDS and separated on SDS-polyacrylamide gel electrophoresis (12.5%) in reduced state were identified by ^{125}I-labeled STA. (upper) Autoradiogram of SDS-polyacrylamide gel electrophoresis of whole platelets stained with ^{125}I-STA. The most intensely labeled bands were GPIIIa followed by GPIV. (lower) Densitogram of autoradiogram (O.D. at 430 nm).

Table 1. Inhibition of STA-induced agglutination

Inhibitor	Concentration	Incubation Time (min)	Agglutination *
—	—	—	+++
EDTA	5mM	2	+++
diltiazem	0.5mM	5	+++
TMB-8	0.5mM	5	+++
PGE_1	5 μM	3	+++
aspirin	100 μg/mℓ	10	+++
trifluoperazin	100 μM	10	+++
iodoacetate	0.5mM		
+		40	+++
antimycin A	2 μM		
GlcNAc	50mM	5	+++
$(GlcNAc)_2$	3mM	5	+
$(GlcNAc)_3$	0.3mM	5	0 ~ ±

Saline was used for dissolving all of the inhibitors except for aspirin and antimycin A. These two inhibitors were dissolved in 99.9% ethanol and diluted with saline (under 0.1% ethanol in final). 490 µℓ of washed platelets was preincubated at 37°C with 10 µℓ of inhibitors for indicated times, prior to STA (100 µg/mℓ).

* Degree of agglutination without inhibitors is expressed as +++. Agglutination is recorded on a scale of 0 to +++. Each experiments were repeated, at least, for three times.

tor; trifluoperazin (100 µM) did not influence STA-induced platelet agglutination (Table 1). These data suggest that STA-induced platelet agglutination does not require metabolic activities and is independent of intracellular metabolism in platelets.

We examined whether or not STA induced platelet activation by measuring the release of platelet granules, TXB_2 formation and platelet protein phosphorylation. WGA (GlcNAc and N-acetylneuraminic acid determinant) was used as a control lectin. STA-induced platelet agglutination was not accompanied by the release reaction (ATP, serotonin, β-TG and PF4 release) or TXB_2 formation (Table 2). The protein phosphorylation in platelets that have been labeled by preincubated with ^{32}Pi is closely related with release reaction (20,21). The phosphorylation of 20K and 40K protein is under the control of Ca^{2+}+calmodulin dependent myosin light chain kinase (21,22) and Ca^{2+}/diglyceride-stimulated, phospholipid-dependent protein kinase C (23), respectively. In our study, STA-induced phosphorylation was almost undetectable, al-

Table 2. Lectin-induced release reaction and TXB_2 formation

	STA (100 μg/mℓ)	WGA (100 μg/mℓ)
ATP *	not detectable	2.18±0.40 μM†
Serotonin **	0.66±0.12%	11.4±0.21%
β-TG ***	0.37±0.10%	22.4±0.89%
PF4 ***	0.50±0.09%	20.3±1.20%
	TXB_2 formation (ng/mℓ)	
Saline (control)	0.26±0.10	
STA (100 μg/mℓ)	0.33±0.07	
WGA (100 μg/mℓ)	11.8 ±1.25	

All values are means±SD of three experiments. * ATP was measured by luciferin-luciferase method as described by Feinman et al. (ref. 15). ** The value was expressed as the percent release of total [^{14}C] serotonin in the platelets. *** The value was expressed as the percent release of total level of β-TG (or PF4) in the samples of 1% Triton X-100 solubilized platelet suspensions. † ATP release induced by WGA, expressed as percentage (mean±SD) of that releasable by thrombin, was 29±6% (n=5).

though WGA induced not only phosphorylation of 20K and 40K protein (Fig. 3) but release reaction and TXB_2 formation (Table 2). Although Con A showed a similar pattern of staining as STA (4), Con A induced release reaction and

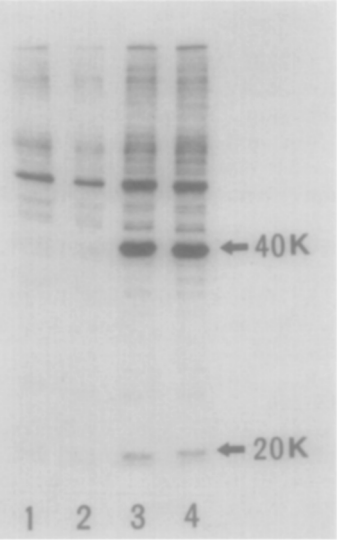

Fig. 3. Autoradiograph of SDS-polyacrylamide gel electrophoresis (12.5%) showing effect of lectins on platelet protein phosphorylation. Samples of a suspension of ^{32}Pi-labeled platelets were incubated 2 min at 37°C with or without lectins. The incubations were terminated by additions of sampling buffer [16.5 mM Tris-HCℓ, 2.3% SDS, 10% glycerol (w/v), pH 6.8] with 5% 2-mercaptoethanol. Lane 1, control platelets; Lane 2, STA (100 μg/mℓ)-stimulated platelets; Lane 3, WGA (100 μg/mℓ)-stimulated platelets; Lane 4, thrombin (0.1 U/mℓ)-stimulated platelets.

platelet protein phosphorylation (5). These data suggest that difference in sugar sequence of lectin-binding sites is one of the factors that determine the presence or absence of platelet activation.

Clinically, the platelets of the patients with Glanzmann's thrombasthenia are deficient in GPIIb/IIIa complex which plays an important role in platelet aggregation (24). The increase of GPIV content was reported in patients with storage pool disease (25) or myeloproliferative disorders (26), although no biological function has yet been ascribed to this glycoprotein. While platelet aggregation study using conventional stimulating agents such as ADP, collagen or arachidonic acid is influenced by many factors such as change of intracellular metabolism or drug intakes (i.e. aspirin), our study suggest that STA-induced agglutination is affected almost only by the membrane abnormality, mainly that of GPIIIa and/or GPIV. It must be emphasized that STA may be used as a simple probe to explore the clinical condition in which the composition of platelet membrane glycoprotein is altered.

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