Short Communication

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Lipid-like Components Predominantly Appeared on High-virulent Trypomastigotes of *Trypanosoma cruzi*

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Abstract: The trypomastigote stage of *Trypanosoma cruzi* plays the most important role in completing infection of mammals. Therefore, the investigation of trypomastigote functions is useful to understand parasite virulence. We have been studying functional difference between trypomastigotes of high and low virulence. In the present work, we examined difference between components separated from living trypomastigotes of respective virulence by trypsin treatment, because trypsin treatment has been known to remove some important functions of trypomastigotes. Separated components were passed through a Benzamidine-Sepharose 6B column to remove trypsin, concentrated and dialyzed against 0.85% saline in a cellulose tube which permits molecules with less than 3, 500 molecular weight (Mr) to pass. A part of concentrated sample was applied to SDS-poly-acrylamide gel electrophoresis in slab gels. The gels fixed in 12.5% trichloracetic acid after electrophoresis showed markedly larger amount of white deposits with a Mr range 37, 000 to 50, 000 on the lanes of high-virulent trypomastigotes than those of low-virulent. The deposits might be dissolved in methanol-acetic acid-water mixtures which were used for a staining sloution and a fixative for the following protein staining, because the part of each lane where the deposits had existed was left unstained. Furthermore, precipitated proteins of concentrated samples by five times volume of acetone showed to have lost the deposits on SDS-PAGE. These results suggested that high-virulent trypomastigotes possessed markedly larger amount of lipid-like components combined to cell surface by protein compared with low-virulent ones.

Key words: Trypanosoma cruzi, Trypomastigote, Surface component, Lipid-like component

The trypomastigote stage of *Trypanosoma cruzi* is the most differenciated in three major developmental stages and plays the most important role in completing infection of mammals. The existence of some functionally indespensable glycoproteins on the cell surface of trypomastigote was shown directly (Snary and Hadson, 1979; Snary, 1983; Nogueira *et al.,* 1981) and indirectly (Kipnis *et al.*, 1981, Henriques *et al.*, 1981). However, the components specifically concerned with virulence have not been determined yet.

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We have been conducting the comparative studies on trypomastigotes of different virulence derived from a single strain (Hermosura *et al.*, 1985; Kanbara *et al.*, 1987a, b). In the present work, we compared components which were separated from the cell surface of living trypomastigotes of different virulence by mild trypsin treatment, based on the fact that trypsin treatment removed important molecules from trypomastigotes, and subsequently transformed them sensitive to alternative complement pathways and macrophage phagocytosis.

Both high- and low-virulent trypomastigotes were derived from the Tulahuen strain, the former were obtained through a CM-cellulose column (Kanbara and Nakabayashi, 1983) from ICR-mouse-skin fibloblast cultures infected with blood trypomastigotes from severely infected mice and the latter were obtained by the same manner from L-cell cultures infected with culture forms maintained in modified NNN medium for a long time (Hermosura *et al.*, 1985). Isolated trypomastigotes (10^8-10^9 in total) were washed three times in phosphate buffered saline (PBS), pH 7.5 by centrifugation at 1,800 *g* for 10 min, suspended in 5 ml of PBS containing 200 μg/ml trypsin (No. F8253, Sigma) and incubated at 37℃ for 30 min. Treated samples were vortexed for 30 sec, and centrifuged at 1,800 *g* for 10 min. The supernatant was filtered through 0.45 μm filter (Milex-HA, Millipore) and stored at −25℃. One hundred ml of stocked supernatant was adjusted to pH 8.0 by adding Tris-HCl buffer (pH 9.0, 1 M) and to 0.5 M NaCl by adding 2.1 g of NaCl and applied to a Benzamidine-Sepharose 6B (Pharmacia) column (1.5 × 7 cm) to remove trypsin, which was previously equilibrated by 50 mM Tris-HCl buffer (pH 8.0) containing 0.5 M NaCl. Elution was done using the same buffer at a flow rate of 1 ml/min, and every 5 ml of eluate was collected in a separate tube. Protein of each eluate was measured by Lawry's method. After disappearance of detectable protein, elution buffer was changed to 10 mM HCl containing 0.5 M NaCl to wash out captured trypsin at a flow rate 2.5 ml/min for 30 min and again equilibrated by 50 mM Tris-HCl buffer (pH 8.0) containing 0.5 M NaCl for the next use. Eluate containing protein was concentrated in a cellulose tube (Dialyzer Tubing, M.W. Cut off 3,500, Nakarai Chemicals LTD) against polyethylen glycol 20,000 and dialysed to 0.85% saline. A part of concentrated eluate was mixed with equal volume of 0.125 M Tris-HCl (pH 6.8) containing 20% glycerol, 10% 2-mercaptoethanol and 4.6% sodium dodecyl sulfate (SDS), boiled for 2 min and subjected to SDS- polyacrylamide gel electrophoresis (SDS-PAGE), which was carried out in 12.5% slab gels (acrylamide : bis-acrylamide = 37 : 1), using 5% gels for stacking. The molecular weight standards ranging from 14,400 to 92,500 (Bio-Rad) were used as markers. After electrophoresis, gels were first fixed in 12.5% trichloracteic acid (TCA) for 30 min, which showed markedly larger amount of white deposits on the lanes of the eluate from high-virulent trypomastigotes than from low-virulent ones (Fig 1 A). These deposits had wide range of molecular weight from 37,000 to 50,000 and were conceived to be prominent components in high-virulent trypomastigote eluate judging from protein staining pattern which were shown by the following staining with Coomassie Brilliant Blue R and Silver stain, i. e. only a few proteins were detectable by the former

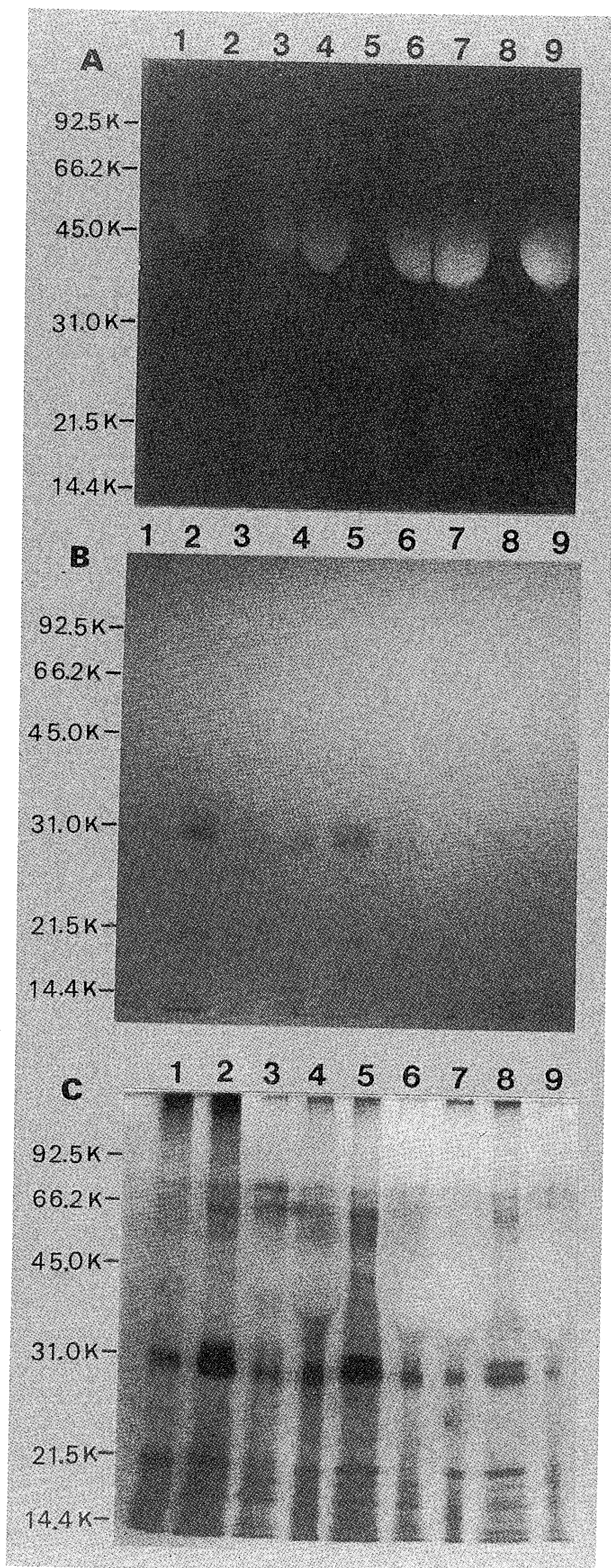

Fig. 1.

A : Separated components from living trypomastigotes by trypsin treatment were applied to SDS-PAGE and them fixed in 12.5% TCA. lane 1: separated components from low-virulent trypomastigotes, lane 2: precipitated samples by acetone of those from low-virulent ones, lane 3: precipitated samples by TCA of those from low-virulent ones, lane 4 and 7: separated components from high-virulent trypomastigotes obtained in different time, lane 5 and 8: precipitated samples by acetone of the same as lane 4 and 7 respectively, lane 6 and 9: precipitated samples by TCA of the same as lane 4 and 7 respectively.

B : The same arrangement of the samples stained with Coomassie Brilliant Blue R.

C : The same arrangement of the samples stained with silver stain.

and numerous by the latter. Surprisingly, the parts where white deposits existed were left unstained (Fig. 1 B, C). This suggested that the deposits were dissolved and removed from the gels by the solution for Coomassie (50% methanol and 10% acetic acid in water) or fixative for silver stain (40% methanol and 10% acetic acid in water). Sugar staining of the deposits by periodic acid-Schiff after TCA fixation was negative (data not shown). Furthermore, the concentrated eluate was mixed with five times volume of acetone, and precipitate collected by centrifugation at 1,800 *g* for 15min was dissolved in 0.0625 M Tris-HCl (pH 6.8) containing 10% glycerol, 5 % 2-mercaptoethanol and 2.3% SDS, boiled for two min and subjected to SDS-PAGE. The white deposit by 12.5% TCA disappeared after aceton treatment and new protein bands appeared (Fig. 1. A, C; lane 2, 5 and 8), whereas precipitated samples by TCA treatment retained them (Fig 1; lane 3, 6 and 9). These results suggested that the deposit consisted of mainly lipid-like molecules combined with proteins. At present the function of lipid-like components is unknown but it is notified that remarkably bigger amount of them were found on high-virulent trypomastigotes compared with low-virulent ones. They are combined to the cell surface by protein (or peptids) because trypsin treatment removed them. Connelly and Kierszenbaum (1984 and 1985) reported that phospholipase A or D treatment of the parasite surface enhanced parasite-host cell association between *T. cruzi* trypomastigotes and host cells including phagocytic and nonphagocytic cells. This result also suggested that lipid-like component containing phospholipids might exist on the cell surface although they conceived phospholipase treatment might act to membrane phospholipids and change membrane composition or physiology. They used blood trypomastigotes of the Tulahuen strain from infected mice which is corresponding to high-virulent trypomastigotes in the present work. Therefore increase in parasite-host cell association by phospholipase treatment may explain the reason for faster uptake by macrophages of low-virulent trypomastigotes (Hermosura *et al.*, 1985) bacause they possess markedly less amount of lipid-like components and parasite virulence.

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Trypanosoma cruzi の強毒株 trypomastigote 細胞表面には脂質様成分が優勢に存在する

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Trypanosoma cruzi の trypomastigote stage はこの原虫の哺乳類への感染に最も重要な役割を果す。従ってこの原虫の病原力の解明にはこの stage の原虫のもつ機能を追及するのが最も良い方法だと思われる。これまで同一株由来の病原力の異る trypomastigote のもつ機能の比較研究を行ってきたが、ここでは生きた trypomastigote がトリプシンの作用で2～3の重要な機能を失うことより、このトリプシン作用で生 trypomastigote から遊離される成分について病原力の異るものの差異を検討した。遊離成分は Benzamidine-Sepharose 6B のカラムを通しトリプシンを除いた後に分子量3,500以上を通過させないセルロ-スチュ-ブを用いて濃縮して SDS-PAGE にて分析した。泳動後12.5% TCA 固定にて強毒株由来遊離成分中に著名に多量の白濁沈殿部を認めた。この沈殿物はその後の蛋白染色では消失することから、メタノ-ル、酢酸を含む染色溶媒または蛋白固定液中に溶解すると考えられる。更に5倍量のアセトンを用いてトリプシンによる遊離成分中の蛋白を沈殿させ、SDS を含む溶解液で溶解後 SDS-PAGE にかけると、沈殿部分は消失する。これらのことから強毒株 trypomastigote 細胞表面には、蛋白によって結合された脂質様成分が弱毒のそれに比し著明に多量に存在することが予想される。

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