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Trypanolytic Factor and Its Inhibitor in Normal Guinea Pig Serum

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Abstract

The trypanolytic factor and its inhibitor in guinea pig serum was studied under experimental conditions by enhancing the trypanosome lytic activity. The activity of the factor was suppressed completely in normal serum. Gel filtration with Sephadex G-200 or chromatography using DEAE-Sepharose suggested that the suppression might be caused by the binding of inhibitor to the factor. The relative molecular weight of the resulting complex was estimated to be 3×10^5. The trypanolytic factor was stable at 56°C, whereas the inhibitor was labile. The main component responsible for lysis of trypanosomes was suggested to be a protein by chemical and enzymatic characterization. The inhibitor was a small molecule of relative molecular weight lower than 5×10^3; it was inactivated by treatment with neuraminidase. The heat-inactivated (56°C, 30 min) FBS (10%) was used throughout the experiments as a supplement to enhance trypanosome lytic activity. However, FBS itself could not overcome the suppression mediated by the inhibitor in normal serum.

Key words: Protozoa, *Trypanosoma brucei gambiense,* Trypanolytic factor, Inhibitor, Guinea pig serum, Fetal bovine serum

Introduction

What determines the host range of parasitic infections has long been a fundamental question in parasitology. The natural immunity of the host has been suggested to be a factor in restricting the infectivity of parasites. In this respect, much attention has been focussed on the finding of Laveran (1902) that normal human serum contains trypanosome lytic activity specific to *Trypanosoma brucei brucei* in blood stream. The material responsible for the activity was first identified as high-density lipoprotein (HDL) by Rifkin (1978b). Recently, Hajduk *et al.* (1989) purified the lytic activity about 8,000-fold and proposed the precise molecular profile of the material responsible for the activity. They showed that trypanosome lytic activity is associated with a minor subclass of HDL which contains five classes of apolipoprotein and at least three unique proteins.

T.b.brucei is infective to many laboratory animals. Rifkin (1978b) indicated that HDL of rat or rabbit has no trypanocidal activity against *T.b.brucei.* In contrast, it was reported by D'Hondt and Kondo (1980) that guinea pig and mouse sera can exhibit trypanosome lytic activity against *T.b.brucei* or *T.b.rhodesiense* in the presence of glycerol. It remains questionable whether loss of resistance to trypanosome infection is due a reduction in the quantity or activity of the trypanosome lytic factor in animal sera.

The Wellcome strain of *Trypanosoma brucei gambiense* in blood stream is susceptible to the lytic activity of human serum, but show the resistance against guinea pig serum. This study investigated the factor in guinea pig serum inhibiting lysis of this strain of *Trypanosoma.*

Material and Methods

Sera. Guinea pig sera (GPS) were prepared from pooled blood obtained by cardiac puncture

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from normal guinea pigs of a conventional strain weighing about 300 g (Japan SLC Inc., Sizuoka, Japan). Mouse and rat sera were separated from pooled blood obtained by cardiac puncture from normal animals aged 13 weeks of the BALB/c and SD strains (Charles River Japan, Inc., Kanagawa, Japan). Rabbit serum was prepared from pooled blood obtained by venipuncture from normal, 19-week-old New Zealand white rabbits (Charles River Japan, Inc.). Horse serum was purchased from the Research Foundation for Microbial Diseases of Osaka University (Osaka, Japan) and fetal bovine serum (FBS) was purchased from Hyclone Laboratories Inc. (Utah, USA). All sera were stored at −20°C.

Trypanosomes. The Wellcome strain of *Trypanosoma brucei gambiense,* obtained from the Department of Parasitology of the Institute for Microbial Diseases in Osaka University (Osaka, Japan), was maintained in BALB/c mice by passage at 3-day intervals and used as the target organism for the lytic action of GPS. Trypanosomes were harvested from mice 72 hr after infection, separated from blood by passage through a DEAE-cellulose column according to Lanham and Godfrey (1970), washed, and resuspended in RPMI 1640-1 medium (pH 7.0, Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) at 2×10^6 trypanosomes per ml.

Trypanosome lytic activity (TLA). Fifty microliters of GPS was serially diluted with an equal volume of RPMI 1640-1 in a Falcon 3072 tissue culture plate with 96 flat-bottomed wells (Becton Dickinson Labware, New Jersey, USA). Fifty microliters of trypanosome suspension (2×10^6/ml) was added to each well. A series of test wells was accompanied by a control well containing medium without GPS. Heat-inactivated FBS (56°C, 30 min), as a supplement, was added to each well at a final concentration of 10%. In some experiments, 145 mM glycerol was added to wells instead of 10% FCS. Plates were incubated at 37°C for 1 hr under 5% CO_2. After incubation, the viability of the trypanosomes was assessed by microscopical observation. The final dilution showing almost complete lysis of trypanosomes was taken as the end-point titer of TLA of the sample. Each test was performed in duplicate, and the higher titer was taken as the titer of the sample.

Treatment of GPS by heat or dialysis. Microtubes (Sarstedt, Germany) containing 150 μl of sera were heated in a water bath. GPS heated at 56°C for 30 min is referred to H-GPS. Dialysis of whole sera was performed several times against excess volumes of phosphate-buffered saline (pH 7.0, PBS-7) at 4°C for one week, using cellulose tubing (Viskase Sales Corp., Illinois, USA).

Treatment of GPS with chemical reagents. Trichloroacetic acid (TCA) was added to 1 ml of H-GPS to a final concentration of 2.5%. After standing at room temperature for 30 min, the supernate was separated by centrifugation (10,000 rpm, 20 min) and dialyzed several times against a total volume of 3 liters of PBS-7 at 4°C for 24 hr.

Five milliliters of H-GPS was mixed with the same volume of saturated ammonium sulfate solution. Precipitates were separated from the supernates by centrifugation (3,000 rpm, 4°C, 30 min), washed and redissolved in 5 ml of water. To eliminate ammonium sulfate, both the supernate and solution of redissolved precipitate were dialyzed extensively against PBS-7 before assay of TLA.

pH-stability. One milliliter of H-GPS was dialyzed at 4°C for 24 hr against 200 ml of RPMI 1640-1 medium at pH 3, 4, 5, 6, 8 or 9. All sera were then centrifuged (10,000 rpm, 4°C, 1 hr) and the pH of the supernates returned to 7.0 by dialysis against an excess volume of RPMI 1640-I at pH 7.0.

Ultrafiltration. Two kinds of filters, UFP1 LCC24 with nominal molecular weight limit (NMWL) of 5×10^3 and UFP1 THK24 with NMWL 1×10^5 (Nihon Millipore Kogyo K.K., Yonezawa, Japan) were used. One milliliter of intact GPS was passed through the filter by air pressure. The recovery of ultrafiltrate from the

NMWL 5×10^3 filter (fraction II) was 70–80%. The fraction remaining on the NMWL 1×10^5 filter was reconstituted with 1 ml RPMI 1640-1 by rinsing (fraction I).

Assay of inhibitory activity. Twenty-five microliters of intact GPS, ultrafiltrates or medium was mixed with 25 μl of serial dilutions of H-GPS before trypanosomes were added. The TLA assay was performed under standardized conditions.

Enzymatic treatments. Fraction I, containing the TLA factor, and fraction II, containing the inhibitor, of the ultrafiltrate of GPS were digested with proteases and neuraminidase. As proteases, insoluble trypsin and proteinase K were used. All enzymes used were purchased from the Sigma Chemical Company (Missouri, USA). Five milligrams insoluble trypsin, 0.1 mg proteinase K or 50 μg neuraminidase were allowed to react with 1 ml of each fraction in a microtube incubated at 37°C with gentle shaking. The incubation time of fraction I with proteases was 1 hr and that with neuraminidase was overnight. The incubation period of fraction II with proteases was extended to overnight and that with neuraminidase was shortened to 1 hr as a consequence of preliminary observations. The insoluble trypsin was removed from digests by centrifugation. Fraction I, after digestion with proteinase K, was treated with small volumes of 250 units of urinastatin (Mochida Pharmaceutical Co., Tokyo, Japan), a protease inhibitor. Fraction I, after digestion with neuraminidase, was assayed without any additional treatment. Proteinase K and neuraminidase were removed from digests of fraction II by ultrafiltration using an NMWL 5×10^3 filter.

Gel filtration. One milliliter of native GPS was fractionated at room temperature using a 2×150 cm column of Sephadex G-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) buffered with PBS-7. The flow rate of PBS-7 was 0.25 ml per min and the fraction volume was 3 ml. The protein concentration of each fraction was plotted by photometrical absorbency (OD) at 280 nm. Fractions were pooled appropriately for economical assay of TLA. Each pool was tested for TLA before or after heating at 56°C for 30 min.

Chromatography. DEAE-Sepharose CL-6B was purchased from Pharmacia Fine Chemicals and equilibrated with potassium phosphate buffer (KPB, 0.02 M, pH 6.8). A column of 1×20 cm was used for 15 ml native GPS dialyzed against KPB. After application of GPS, the column was washed with 300 ml of KPB. Elution was performed at room temperature using 3,000 ml of KPB with a linear concentration gradient of 0 to 0.15 M NaCl. The flow rate of buffer was 1.8 ml per min and the fraction volume was 2.5 ml. The protein concentration of each fraction was estimated photometrically by means of OD at 280 nm and plotted. The content of neutral sugar of each fraction was assessed by phenol reaction followed by determination of OD at 490 nm. Every 10 fractions were tested for TLA before or after heating at 56°C for 30 min; fractions showing TLA were pooled and dialyzed against starting buffer (KPB) before the second chromatography using the same sized column of DEAE-Sepharose CL-6B. In this case, the column with the sample applied was washed with 200 ml of KPB and elution was performed in one step using KPB containing 0.025 M NaCl. Other conditions were the same as those for the first chromatography.

Phenol reaction. A 0.2-ml sample was mixed with 1 ml of 4.21% phenol in a glass test tube and 2.5 ml of concentrated sulfuric acid was added. After shaking, the reaction mixture was left to stand at room temperature for 1 hr. The concentration of resulting chromogen was assayed photometrically at 490 nm. The neutral sugar content of fractions was estimated using a standard curve for glucose.

Results

Trypanosome lytic activity (TLA). guinea pig serum after heat treatment at 56°C for 30 min (H-GPS) exhibited TLA *in vitro*. Although this activity was weak, the addition of heat inactivated

Table 1 Effect of supplementary factors on trypanosome lytic activity (TLA)*

	Supplementary Factors			
	–	Glycerol (145 mM)	FBS† (10%)	Dialyzed FBS† (10%)
Intact GPS	0	0	0	0
GPS heated at 56°C for 30 min	2^1	2^1	2^5	2^1

*TLA was expressed as the reciprocal of the serum dilution necessary to induce complete lysis of trypanosomes under standardized conditions with or without supplementary factors.
†FBS heated at 56°C for 30 min.

(56°C, 30 min) fetal bovine serum (FBS) considerably augmented it. However, the FBS itself did not activate intact guinea pig serum (GPS). Glycerol did not affect TLA of both intact and heated GPS (Table 1). In our subsequent studies on TLA in GPS, 10% FBS was used as a supplement. FBS at this concentration was not toxic to trypanosomes and its supplementary effect was mediated by unidentified components with small molecular size dialyzable through a cellulose membrane (Table I). TLA was present in GPS heated at 50°C for 1 hr and reached a maximum in GPS heated at 56°C for 30 min. The results demonstrated that TLA was mediated by a serum component (TLA factor) which is stable to heating at 65°C for 30 min (Fig. 1). The same degree of TLA was observed in GPS subjected to prolonged dialysis against PBS-7 using cellulose tubing (Fig. 1). TLA was not detected in sera from horse, rabbit, rat or mouse even after heating or dialysis (Table 2).

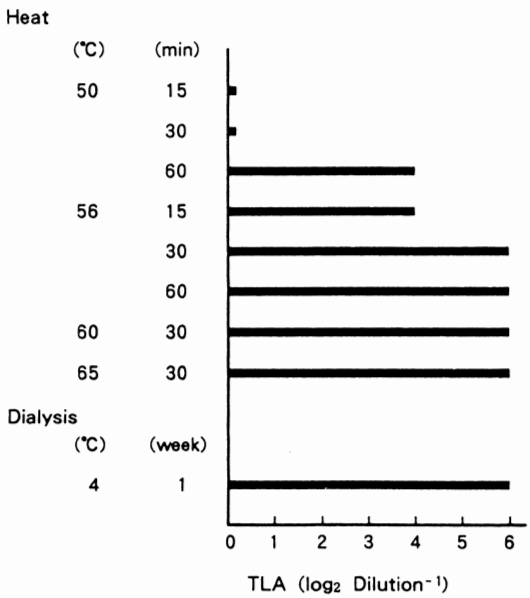

Fig. 1 Appearance of trypanosome lytic activity (TLA) in guinea pig serum by heat or dialysis. Pooled serum was heated in a water bath for different periods as indicated in the figure. The dialysis of pooled serum was performed against PBS-7 at 4°C for 1 weeks. TLA is expressed as reciprocal of serum dilution necessary to induce complete lysis of trypanosomes under standardized assay condition.

Temperature dependency of trypanolytic reaction. In order to determine the effect of temperature on the trypanolytic reaction of H-GPS, 1×10^5 trypanosomes were incubated for

Table 2 Trypanosome lytic activity (TLA)* of sera from various animals

	intact serum	heated serum (56°C, 30 min)
Guinea pig	–	+
Mouse	–	–
Rabbit	–	–
Rat	–	–
Horse	–	–

*Sera were tested for TLA at a concentration of 50% under standardized conditions with 10% heat-inactivated FBS.

1 hr at various temperatures in 100 μl of RPMI 1640-1 containing 10% FCS and serially diluted H-GPS. As shown in Fig. 2, the lysis of trypanosomes by H-GPS was temperature-dependent and maximal at 37°C.

Characterization of TLA factor. Results were shown in Table 3. Treatment of H-GPS with TCA at 2.5% of final concentration completely eliminated TLA from the supernate. Precipitates were insoluble and TLA could not be recovered. Ammonium sulfate at 50% final concentration precipitated TLA factor from H-GPS. TLA was not detected in the supernate, but recovered by redissolving the precipitates. The recovery of TLA was about 25% of that of the original H-GPS. H-GPS was left to stand for 24 hr at 4°C at pH values ranging from 3 to 9. The pH of supernates of high-speed centrifugation was returned to 7.0 before assay of TLA. No change in TLA titer between supernates and original H-GPS was observed. The TLA factor was, therefore, stable for at least 24 hr at pH 3 to 9.

Separation of TLA factor and inhibitor by ultrafiltration. TLA was apparent in GPS only after heating or extensive dialysis. This suggested

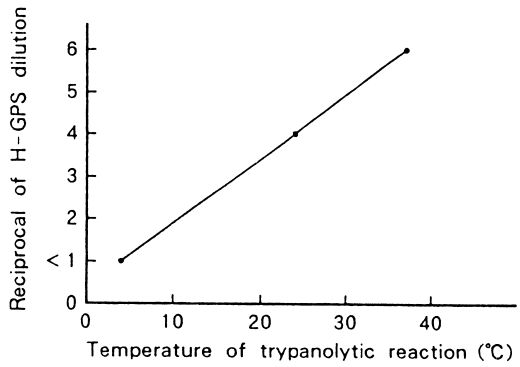

Fig. 2 Concentration of H-GPS required for complete lysis of trypanosomes at various temperatures. H-GPS means guinea pig serum activated by heat at 56°C for 30 min. Trypanosomes (1×10^5) were suspended in 100 μl of medium composed of 50 μl RPMI 1640-1 containing 20% heat-inactivated FCS and 50 μl of serial dilutions of H-GPS in RPMI 1640-1.

the existence of an inhibitor to TLA in intact GPS. The separation of TLA factor and its inhibitor from GPS was attempted using ultrafilters with nominal molecular weight limits of 1×10^5 or 5×10^3 (Table 4). The TLA factor was retained on the NMWL 1×10^5 filter. The factor

Table 3 Characterization of trypanolytic factor in H-GPS*

Treatments	TLA Titer (Log_2 Dilution^{-1})†		
	H-GPS	Supernates	Solution of Precipitates
–	5		
2.5%‡ CCl_3COOH		0	–‖
50%‡ $(NH_4)_2SO_4$		0	3
pH-Stat§ for 24 hr			
at 3		5	–
4		5	–
5		5	–
6		5	–
7		5	–
8		5	–
9		5	–

*Guinea pig serum heated at 56°C for 30 min.
†Trypanosome lytic activity was expressed as the reciprocal of the dilution of sample necessary to induce complete lysis of trypanosomes under standardized conditions.
‡Final concentration.
§pH-stat at 4°C by dialysis against RPMI 1640-1 at respective pH.
‖Not determined.

Table 4 TLA and inhibitory activity in fractions separated from intact guinea pig serum by ultrafiltration

NMW of fractions	Heat (56°C, 30 min)	TLA‡	Inhibitory activity§
$>1\times10^5$	–	–	–
	+	+	–
$<1\times10^{5\dagger}$	–	–	+
	+	–	–
$>5\times10^{3*}$	–	–	–
	+	+	–
$<5\times10^{3\dagger}$	–	–	+
	+	–	–

*Unfilterable remains on ultrafilters with a nominal molecular weight (NMW) limit of 1×10^5 or 5×10^3. Fractions were reconstituted with RPMI 1640-1 at concentrations of starting sera.
†Filtrates of ultrafilters with respective NMW limits.
‡Trypanosome lytic activity at 50% sample concentration.
§Inhibitory activity suppressible to TLA of H-GPS when mixed equivalently.

recovered by rinsing the filter (fraction I) was inactive unless heated at 56°C for 30 min. The filtrate passing through this filter did not show TLA, but exhibited TLA-suppressive activity when added to equivalent volumes of H-GPS. The inhibitor even passed through an NMWL 5×10^3 filter. When the filtrate through this filter (fraction II) was heated at 56°C for 30 min, the inhibitory activity was lost completely. This demonstrated that the TLA factor is suppressed in native GPS by interaction with an inhibitor which is heat-labile. The molecular weight of TLA factor after interaction with inhibitor in fraction I was estimated at more than 1×10^5 and that of free inhibitor in fraction II was estimated at less than 5×10^3.

Enzymatic treatment. For the further characterization of TLA factor and inhibitor, fractions I and II of the ultrafiltration were digested with two kinds of proteases and neuraminidase as described in Materials and Methods. The content of TLA factor of fraction I and that of inhibitor of fraction II were assumed to be almost equivalent to those in native GPS. Insoluble trypsin was removed by centrifugation from all digests, but proteinase K and neuraminidase were not removed from digests of fraction I. Therefore, 250 units of urinastatin were added to the proteinase K digest to inhibit enzymic activity. Although preliminary experiments indicated that this concentration of urinastatin did not affect the viability of trypanosomes, the assay of TLA in this digest was performed with controls containing the same amount of urinastatin. The maximal concentration of neuraminidase carried over to the assay wells was 25 μg per ml. This concentration of the enzyme had been previously shown not to affect the viability of trypanosomes. Fraction I showed a TLA titer of 2^4 before enzymic treatment. Digestion of this fraction with trypsin completely abrogated the activity. Digestion with proteinase K also decreased the titer to 2^2, whereas treatment with neuraminidase did not affect the activity. These results are expressed as percentages of the control valve in Table 5. In the case of digests of fraction II, proteinase K or neuraminidase were removed by ultrafiltration using a NMWL 5×10^3 filter. Before the enzymatic treatment, fraction II completely inhibited the lysis of trypanosomes by H-GPS at a TLA titer of 2^5, when both were mixed in equivalent volumes. As shown in Table 6, the treatment of this fraction with neuraminidase eliminated the inhibitory activity thoroughly while the treatment

Table 5 Enzymatic treatments of fraction (NMW $> 1 \times 10^5$) separated from intact guinea pig serum by ultrafiltration

Treatments	TLA (% of control)[§]
Trypsin*	0
Proteinase K[†]	25
Neuraminidase[‡]	100

*Treatment with 5 mg/ml insoluble trypsin at 37°C for 1 hr.
[†]Treatment with 0.1 mg/ml proteinase K at 37°C for 1 hr.
[‡]Treatment with 50 μg/ml neuraminidase at 37°C overnight.
[§]Trypanosome lytic activities assayed after heating (56°C, 30 min). TLA of untreated fraction, assayed after heating, was expressed as 100%.

with proteases did not affect the inhibitory activity.

Gel filtration and Chromatography. Separation of the TLA factor was attempted by gel filtration and chromatography. The fractionation pattern of 1 ml of GPS on a Sephadex G-200 column was plotted by means of absorbency at 280 nm (OD) (Fig. 3). Before testing for TLA, fractions were pooled as shown in the Figure. None of the pools showed TLA until heated at 56°C for 30 min. After heating, TLA was detected in pool No. 3 only. This indicated that the TLA, the activity of which was suppressed, was filtered out slightly faster than IgG class immunoglobulin. Referring to the positions of immunoglobulins and albumin in Fig. 3, the relative molecular weight of TLA factor, probably as a complex with inhibitor, was estimated by interpolation as 3×10^5. The separation of TLA factor in GPS was next attempted by repeated chromatography using DEAE-Sepharose CL-6B under the conditions described in Materials and Methods and the legend to Fig. 4. At the first chromatography (Fig. 4A), the bulk of the immunoglobulins was washed out with starting buffer (KPB) before the elution of TLA factor. The elution pattern of proteins remaining on the column after washing was determined by plotting the OD at 280 nm. The neutral sugar content of each fraction was assessed by the OD at 490 nm after phenol reaction. The base line indicates a sugar content of less than 0.18 μg per ml of glucose. The hatched area indicates

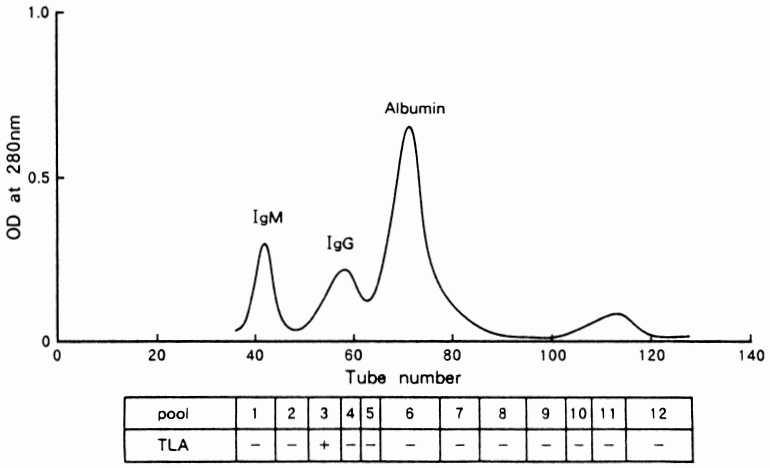

Fig. 3 Gel filtration of 1 ml intact guinea pig serum on a 2×150 cm Sephadex G-200 column. The following conditions were used; buffer, PBS-7 containing 0.03% sodium azide; fraction size, 3 ml; flow rate, 0.25 ml/min; temperature, room temperature. Fractions were pooled as shown in the figure. A small volume of each pool was tested for TLA before or after heating at 56°C for 30 min under standardized conditions. TLA was negative in any pools before heating.

Table 6 Enzymatic treatments of fraction (NMW $< 5 \times 10^3$) separated from intact guinea pig serum by ultrafiltration

Treatment	Inhibitory activity (% of control)§
Trypsin*	100
Proteinase K†	100
Neuraminidase‡	0

*Incubation with 5 mg/ml insoluble trypsin at 37°C overnight.
†Incubation with 0.1 mg/ml proteinase K at 37°C overnight.
‡Incubation with 50 μg/ml neuraminidase at 37°C for 1 hr.
§Inhibitory activity of untreated fraction to suppress completely lyses of trypanosomes by equivalent volume of H-GPS was expressed as 100%.

fractions showing TLA, which was assayed after the fractions had been heated at 56°C for 30 min. In order to remove the slight amount of contaminating proteins from the recovered eluate showing TLA, the column used in the second chromatography of this eluate was washed thoroughly with starting buffer before elution, and TLA factor was eluted thereafter in one step using the NaCl concentration calculated from Fig. 4A to cut off slow-moving proteins. Concentrations of protein and neutral sugar in the eluate showing TLA were of the order of base line values (Fig. 4B). The TLA factor isolated finally was subjected to three overnight dialyses (two against KPB, one against PBS-7) and two chromatographies before the assay of TLA. However, its TLA was still suppressed, and only heating the eluate at 56°C for 30 min permitted expression of the activity. The TLA factor isolated in PBS-7, without the other components of GPS, was very unstable and lost its activity after a few days even at 4°C.

Discussion

The Wellcome strain of *Trypanosoma brucei gambiense* is infective to many laboratory animals. Sera from these animals were screened for resistant properties to infection with this *Trypanosoma*. No resistant activity was detected

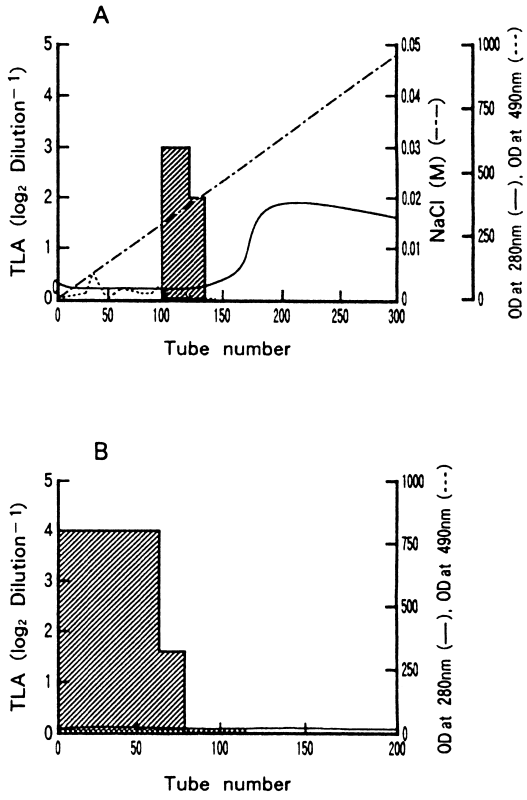

Fig. 4 Purification of trypanosome lytic activity (TLA) in guinea pig serum by chromatography using DEAE-Sepharose CL-6B equilibrated with potassium phosphate buffer (0.02 M, pH 6.8, KPB). Protein concentrations in fractions were expressed as optical density (OD) at 280 nm (———). Neutral sugar contents of fractions were expressed as OD at 490 nm of phenol reaction (·····). The concentration of neutral sugar calculated in terms of glucose was less than 0.18 g/ml on the baseline. NaCl gradient is shown by dotted line (—·—). Every ten fractions were tested for TLA before or after heating at 56°C for 30 min. No activity was detected before heating. Locations of TLA detected after heating of fractions are shown with titers by hatched areas. A, 15 ml of intract GPS dialyzed against KPB was applied to a 1×20 cm column stood at room temperature. The column was washed with 300 ml KPB before elution using 3,000 ml KPB with a linear concentration gradient of 0 to 0.15 M NaCl. Flow rate was 1.8 ml/min and fraction size was 2.5 ml. B, Rechromaography of TLA recovered by the first chromatography. Fractions shown in the hatched area in Fig. 4A were pooled, dialyzed against KPB and applied to the same column as that in Fig. 4A. After washing with 200 ml KPB, TLA was eluted with KPB containing 0.025 M NaCl.

in intact sera. It was known, however, that GPS exhibited trypanosome lytic activity (TLA) after having been heated at 56°C for 30 min or extensively dialyzed against PBS-7. This suggested that TLA might be suppressed by an inhibitor in intact sera.

D'Hondt and Kondo (1980) reported that addition of 145 mM glycerol augmented the TLA of human serum against *T.b.brucei* or *T.b. rhodesiense,* and induced intact sera from guinea pig or mouse to exhibit TLA. In our study, however, their results could not be reproduced.

The augmentation of TLA of H-GPS by 10% FBS was remarkable. As FBS at this concentration was not toxic to trypanosomes, the augmentation did not appear to be due to a simple additive effect of toxicities of both sera. This activity of FBS seemed to be due to components of small molecular size, because dialysis of FBS using cellulose tubing eliminated the activity completely (Table 1).

Recently, the resistant factor in human serum to *Trypanosoma equiperdum* was identified by Verducci *et al.* (1989). This factor was quite different from the trypanolytic factor identified by Rifkin (1978b) and was an IgM immunoglobulin. The TLA factor of GPS observed in our study was not an immunoglobulin, for the following reasons. 1. The lysis of trypanosomes was induced under conditions in which complement would have been inactivated by heat. 2. The relative molecular mass of TLA factor estimated by gel filtration was quite different from that of IgM and seemed to be slightly higher than that of other classes of immunoglobulin (Fig. 3). 3. TLA factor was separated from most immunoglobulins by chromatography using DEAE-Sepharose (Fig. 4).

The molecule in normal human serum responsible for the specific lysis of *T.b.burcei* was first isolated in the HDL fraction by Rifkin (1978b). Further investigation by Hajduk *et al.* (1989) elucidated a more precise profile of this molecule. The HDL responsible for trypanosome lysis was shown to be a molecular complex with relative molecular weight of 49×10^4 and composed at least of five classes of apolipoprotein and three unique proteins. It was suggested that two kinds of apolipoprotein and two kinds of protein might be related to the activity. The molecular profile of the TLA factor of GPS is still obscure, so that it is impossible to compare both molecules exactly. However, it seems to be reasonable to consider that proteins are important components of the TLA factor of GPS, because (i) treatment of H-GPS with TCA abolished the activity, probably by denaturation of proteins, (ii) treatment with 50% ammonium sulfate precipitated TLA factor, and (iii) treatment with proteases eliminated the activity. The TLA factor of GPS was not inactivated by heat at 65°C (Fig. 1). This point seems to differ from previous observations on human TLA factor (Rifkin 1978a).

It is considered possible that the suppression of TLA in intact GPS is due to interaction with the inhibitor, because the activity of H-GPS was suppressed by the addition of intact GPS (data not shown) or ultrafiltrates of this serum (Table IV). The inhibitory activity of ultrafiltrates was abrogated by heat (Table 4). The molecular weight of inhibitor was estimated to be less than 5×10^3. The interaction of inhibitor with TLA factor seemed to be relatively permanent. The lytic activity of TLA factor separated from intact GPS by gel filtration or chromatography was still suppressed. The mechanism of the interaction is still unclear. The inhibitory activity of ultrafiltrate (NMWL 5×10^3) was mediated probably by free inhibitor. This activity was eliminated completely by treatment with neuraminidase (Table 6). On the other hand, the TLA of H-GPS was abolished when H-GPS was incubated with packed red blood cells from various animals (data not shown). Sialic acids are common components of the cell surface of erythrocytes; sialic acid moieties of the inhibitor may play an important role in interaction between TLA factor and inhibitor.

During the lytic reaction, H-GPS caused swelling of the trypanosomes before lysis. The lytic reaction was temperature-dependent (Fig. 2). These observations are compatible with previous reports of lysis of *T.b.brucei* by normal human serum (Rifkin 1978a, 1984). The action of the trypanolytic factor in human serum was sug-

gested to be catalytic rather than stoichiometric (Rifkin 1984). It was also suggested by Rifkin (1983) that the binding of human serum factor (HDL) to trypanosomes might be more similar to the binding of low density lipoprotein (LDL) to erythrocytes, which is probably due to non-ionic adsorption (Hui *et al.* 1981), than to the LDL-fibroblast receptor model. This suggestion may imply the participation of nonspecific hydrophobic force by lipid components in binding mechanisms of human HDL to trypanosomes. Although the isolation of pure molecules and detail analysis of molecular compositions remains to following studies, our preliminary observations that this TLA factor could be adsorbed by erythrocytes from various animals (data not shown) and TLA was detected in heavy densiy fraction but not in LDL or VLDL fractions of GPS (data not shown) may indicate similarities in the molecular compositions and the binding mechanisms of both guinea pig and human trypanolytic factors.

Our study demonstrated that TLA factors exist not only in sera of primates but also in GPS, and that the activity is, however, suppressed in the latter, the identification of various molecules bearing lytic or inhibitory activities in animal sera will provide some knowledge of mechanisms acting as natural immunity of host against parasitic infection.

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