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Production of Human Type Glycosylated Tissue Plasminogen Activator and the Role of Its Carbohydrate Moiety

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ABSTRACT

To begin the examination of the role of human type carbohydrate moiety of tissue plasminogen activator (t-PA) on the binding of the enzyme to fibrin, the naturally glycosylated enzyme was produced by microcarrier culture of human cells established from normal uterine muscle. The cells grown on microcarriers in Hanks' MEM supplemented with 10 % FBS (1.6×10^6 cells/ml) rapidly detached themselves from microcarrier in a serum-free medium (t-PA production medium) within 5 days, and it was difficult to produce t-PA for long time (t-PA production: an average of 3 IU/ml/day over 5 days). Addition of 0.5% beef extract to the serum-free medium suppressed their detaching from microcarriers. By regulating the pH (7.4) and dissolved oxygen (4 ppm) of the serum free medium, the cell density of microcarrier culture increased to 1.2×10^7 cells/ml and t-PA was produced over 38 days (t-PA production: an average of 836 IU/ml/day over 38 days). Native t-PAs purified to homogeneity from the culture broth had the molecular weight of 63,000 and 65,000 containing 5.6 and 8.5 % carbohydrate, respectively (molecular mass of protein moiety was calculated to be 59,500). By enzymatic digestion of carbohydrate moiety in native t-PA, we obtained partially deglycosylated t-PA with molecular weights of 60,000 and 62,000. Completely deglycosylated t-PA was obtained by t-PA production in the presence of 10 μg/ml tunicamycin (*N*-glycosylation inhibitor). This suggests that *N*-glycosylation of t-PA occurs while *O*-glycosylation is absent. The binding strength of these enzymes to fibrin increased with decrease of the carbohydrate content. The carbohydrate moiety of human type glycosylated t-PA probably modulates the binding strength of the enzyme to fibrin.

Keywords: Tissue plasminogen activator, cell culture, carbohydrate, animal cell, fibrinolysis

INTRODUCTION

Tissue plasminogen activator (t-PA) is a serine protease found in nearly all animal tissues and many body fluids.[1] The enzyme enzymatically converts plasminogen into plasmin which lyses fibrin, a main protein component of blood clots.[2] T-PA has the stronger affinity for fibrin than the other plasminogen activators such as urokinase,[3] and is activated by fibrin markedly while other plasminogen activators are not.[4] Thus, t-PA activates plasminogen effectively in the presence of fibrin.

Since the content of the enzyme in human tissues and body fluids is very low, t-PA obtained from recombinant Chinese hamster ovary (rCHO) cells[5] is used clinically in the treatment of acute myocardial infarction.[6] However, the t-PA synthesized by rCHO cells is glycosylated in a manner similar but not identical to the enzyme secreted by human tumor cells (melanoma cells).[5] Since two of the three glycosylation sites of t-PA are located at Asn^{117} and Asn^{184} in the kringle domains (residuces 92-173 and 180-261) thought to be essential for the affinity of t-PA to fibrin,[7] the structure of the carbohydrate side chains probably affects the binding of the enzyme to fibrin. Hansen, *et al.* reported that nonglycosylated t-PA obtained from rCHO cells bound to fibrin more strongly than the glycosylated form,[8] whereas nonglycosylated t-PA obtained from melanoma cells have the same binding properties as the glycosylated form.[9] Thus, the structure and function of carbohydrate moiety of t-PA are in accordance with the type of the t-PA producing cells. The role of the carbohydrate moiety of native t-PA should be examined with the human type glycosylated enzyme secreted by human cells, which are not tumor cells.

The production of t-PA by cell culture without recombinant DNA technique is markedly low. In addition, tunicamycin-treated cells used for the production of non-glycosylated t-PA are reported to secrete markedly less t-PA than non-treated cells.[10] Therefore, the culture conditions for the effective production of naturally glycosylated t-PA should be established.

To examine the role of carbohydrate side chains of human type glycosylated t-PA, we prepared several t-PAs with different carbohydrate contents. In this paper, we describe the production methods of naturally glycosylated t-PA with an established human cell line, and the comparison of its binding strength for fibrin with that of deglycosylated t-PA.

MATERIALS AND METHODS

Materials

Eagle's minimum essential medium with Hanks' salts (Hanks' MEM), 199 medium with Hanks' salts (Hanks' 199 medium) and fetal bovine serum (FBS) were obtained from Gibco; aprotinin (Trasylol) was from Bayer; beef extract was from Difco; fish extract was from Wako Pure Chemical Industries, Osaka, Japan; monoclonal antibody (ESP-2) against t-PA, which recognizes the active site of t-PA, was from Bioscot, Ltd., Scotland; endo-β-*N*-acetylglucosaminidase H (Endo-H) was from Boehringer Mannheim; tunicamycin (TM) was from Sigma; Cytodex 1, concanavalin A (Con-A)-Sepharose and lysine-Sepharose were obtained from Pharmacia; and t-PA International Standard (lot., 83/517) was from WHO. Fibrin-Sepharose was prepared by treatment of human fibrinogen-Sepharose with thrombin.[11] All other chemicals were of highest purity commercially available.

Cells and medium

A human cell line established from normal uterine muscle was obtained from Wakamoto Pharmaceutical Co. (Tokyo). The growth medium was Hanks' MEM supplemented with 10 % FBS. The production medium of t-PA was Hanks' 199 medium supplemented with 10 KIU/ml of aprotinin and 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic asid. Aprotinin was used to avoid conversion of single chain t-PA to the two chain form.[3]

Microcarrier culture

The human cells (5 X 10^7 cells) were inoculated into a 500 ml spinner flask with 150 ml of the growth medium and 1.5 g of Cytodex 1, which was equilibrated in advance with the same medium containing coating agents (10 μg/ml) such as collagen and fibronectin at 37 °C for 1 h. After 2 h at 37 °C with occasional stirring for uniform attachment of the cells to Cytodex 1, the medium was filled up to the final volume (500 ml), and stirred at 40 rpm at 37 °C in an atmospheric air. After the cells grew sufficiently on microcarrieres, the cells with microcarriers were harvested, suspended in 500 ml of the production medium and cultured with stirring at 40 rpm at 37 °C in atmospheric air. 80 % of the medium was harvested and replaced every day with fresh production medium to avoid accumulation of waste materials and enzymatical digestions of t-PA by proteases and gluconosidases.

The microprocessor controlled regulation of pH and dissolved oxygen (DO) in culture were carried out by the sequential addition of 0.3 N NaOH solution or a mixture of four gases (air, O_2, N_2, and CO_2). The gas was supplied through micro-pores of a teflon tube steeped in the medium.

Enzyme purification

T-PA was purified with buffers supplemented with 10 KIU/ml aprotinin and 0.01 % Triton X-100 at 4 °C by the method of Rijken and Collen.[3] The two variants of t-PA were then separated from each other as previously described,[12] with several modifications. Briefly, the t-PA solution was applied to a lysine-Sepharose column (5 X 10 cm) equilibrated with 10 mM phosphate buffer, pH 8.0, containing 0.15 M KSCN. After the column was washed with 1 l of the same buffer, elution was carried out with a linear gradient from 0 to 0.1 M arginine in the same buffer (total volume 800 ml at a flow rate of 100 ml/h, and 8 ml fractions were collected).

Preparation of carbohydrate-depleted t-PA

To remove the carbohydrate side chains of high mannose content from t-PA molecules, t-PA (0.1 mg) was treated with Endo-H (0.1 units) as described previously.[10] After dialysis against 10 mM potassium phosphate buffer supplemented with 1 M NaCl, 10 KIU/ml aprotinin, and 0.01 % Triton X-100, pH 7.5, the enzyme solution was applied to a Con A-Sepharose column (0.55 X 5 cm) equilibrated with the same buffer. Carbohydrate-depleted t-PA was obtained as unadsorbed fraction (10 ml), and hydrolyzed mannose residues were eluted with the same buffer containing 0.4 M methyl α-D-mannopyranoside and 2 M KSCN (10 ml).

Nonglycosylated t-PA was prepared by treatment of the human cells with TM as described previously,[10] with several modifications. Briefly, microcarrier culture of the cells was carried out in the production medium supplemented with TM (5, 10 or 20 μg/ml) and 0.5 % beef extract with regulation of pH (7.4) and DO (4ppm). The culture broth harvested was subjected to a Con A-Sepharose column as described above. The unadsorbed fraction was further applied to a lysine-Sepharose column (1.6 X 5 cm) equilibrated with 10 mM potassium phosphate buffer, pH 8.0, containing 0.15 M KSCN, 10 KIU/ml aprotinin and 0.01 % Triton X-100. After the column was washed with 100 ml of the same buffer, elution was carried out with a linear gradient from 0 to 0.1 M arginine in the same buffer (total volume 100 ml at a flow rate of 20 ml/h, and 1 ml fraction were collected).

Analytical methods

Plating efficiency, one of the important parameters in microcarrier culture,[13,14] was determined by the ratio of colonies formed after cultivation at 37 °C for two weeks to the total number of inoculated cells (200 cells) in 60 mm culture dish with 5 ml of the growth medium. The fibrinolytic activity of t-PA was determined on fibrin gel plates containing plasminogen,[15] and expressed as International Units (IU) with the t-PA International Standard. The specific activity was expressed as IU per mg protein. Protein was determined by the method of Lowry *et al.*[16] with bovine serum albumin as a standard. The living cells and the cells attached to microcarriers were counted with a hemacytometer after trypan blue[17] and crystal violet staining,[18] respectively.

The molecular weight of t-PA was determined by SDS-gel electrophoresis.[19] The carbohydrate content of t-PA was determined by the method of Ranby *et al.*[12] The t-PA activity in the electrophoresed gel was visualized by fibrin zymography.[20]

The strength of binding of t-PA to fibrin was estimated by chromatography with fibrin-Sepharose equilibrated with 0.1 M phosphate buffer containing 10 KIU/ml aprotinin and 0.01 % Triton X-100. T-PA solution (1,000 IU, 1 ml) was applied to a fibrin-Sepharose column (0.5 X 5 cm, 5 mg fixed protein/ml gel). After the column was washed with the equilibration buffer (10 ml), stepwise elution was carried out with 10 ml of the same buffer containing 0.1 M lysine and then the same buffer containing 0.1 M lysine and 1 M KSCN.

RESULTS AND DISCUSSION

Behavior of the human cells

Microcarrier culture is generally suitable for the high density culture of anchorage-dependent cells.

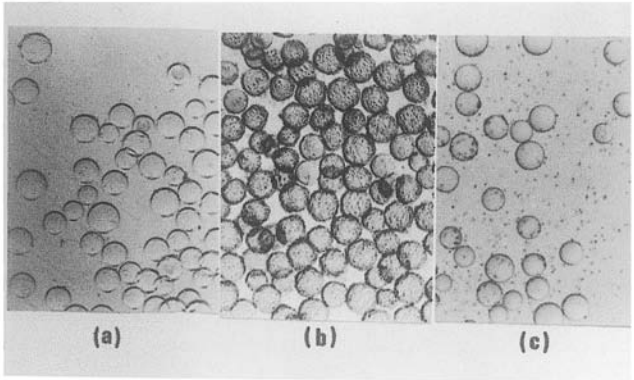

Fig. 1. Attachment, Growth and Detachment of Human Uterine Cells on Microcarrier Culture. The cells were cultured as described in the text. Pictures are the cells attached to microcarriers after cultivation for 2 h (a), grown on microcarriers after cultivation for 6 days (b), and detached from microcarriers after t-PA production for 5 days (c).

Although the culture method has been applied to various cells, the culture conditions vary in accordance with the cell types.[21] To optimize the conditions of microcarrier culture with the human cells, the behavior of the cells in culture was examined. The process of t-PA production by microcarrier culture is divided into two stages: cell growth in a serum-containing medium and t-PA production in a serum-free medium. In the cell growth stage, the plating efficiency of cells is the most important parameter. The plating efficiency of the human cells was approximately 50 % (a relatively high value). Since cells with high plating efficiency are reported to grow from a low inoculation density and to be resistant to agitation,[13,22] the human cells were inoculated with cytodex 1 at a density of 1 X 10^5 cells/ml, and cultured with stirring at 40 rpm (Fig. 1a). After 6 days, KW cells grew uniformly to a density of 1.6 X 10^6 cells/ml (Fig. 1b). Then, the medium was changed to the production medium. Most cells, however, rapidly detached themselves within 5 days from microcarriers in the production medium and died because they do not grow in suspension culture (Fig. 1c). The cells died did not reattach to microcarrier. The TPA produced by the cells attached to microcarriers was an average of 3 IU/ml/day over the first 5 days. The activity (3 IU/ml, 1ml) was completely abolished by incubation with a monoclonal antibody against t-PA (ESP-2, 10 ng) at 4 °C for 14 h, but not with anti-urokinase, suggesting that the human cells secrete t-PA. When the t-PA production was initiated at the half-saturated density of 0.8 X 10^6 cells/ml, the cells grew gradually on microcarriers and detached themselves rapidly. Thus, the cells in the serum-free medium easily detached themselves from microcarrier, and it was difficult to maintain confluent state. The term confluence describes a culture population that occupies all available surface of microcarriers.[23] Therefore, the improvement of cell adhesion was required.

Improvement of cell adhesion

To maintain the human cells on microcarriers for a long time, we examined the effects of several additives and coating agents. After the cells were grown to confluence on microcarriers treated with or without each coating agent, the cells were cultured in the production medium supplemented with each additive. The effects of additives and coating agents were judged on the basis of the period of t-PA production and the average t-PA activity in the medium daily harvested. As shown in Table I, beef extract added to the medium suppressed the detachment of the cells, prolonged markedly the production period, and enhanced greatly the t-PA activity secreted into the medium. The cells secreted t-PA at confluent state for 25 days and then detached themselves gradually. The detachment of

the cells during 25-30 days of t-PA production was probably due to the decrease of cell viability by the repeated culture in the serum-free medium. The increase of t-PA activity is probably due to the suppression of the detachment of the cells, because the t-PA productivity per cells attached to microcarrier was almost equal (about 30 IU/10^6 cells/day) irrespective of the presence of beef extract. The effect of beef extract was not reduced by the removal of dialyzable compounds or by sterilization in an autoclave, although the effective components are not identified at present. Fish extract was also effective but less so than beef extract, and the effective components of fish extract were dialyzable compounds but not macromolecules. The mixture of beef and fish extracts showed the same effect as only beef extract. The other additives and coating agents had no effect.

Regulation of pH and dissolved oxygen

When the cells were cultured in the production medium supplemented with 0.5 % beef extract without regulation of pH and DO, the pH and DO (initially set at 7.4 and 4 ppm, respectively) decreased to 7.0 and almost 0 ppm, respectively, after cultivation for 24 h. Therefore, the effects of pH and DO on the productivity of t-PA were examined in the presence of beef extract (Table II). The effects of pH and DO were judged on the basis of the period of t-PA production, t-PA activity secreted into the medium and t-PA productivity of the cells. Maintaining only pH at a constant value enhanced the

Table I. Effects of Additives and Coating Agents on T-PA Production

Additives (0.5 %)	Coating agents	Period of t-PA production (day)	Average activity (IU/ml/day)
None	None	5	3
Beef extract	None	30	50
Fish extract	None	12	12
Bovine serum albumin	None	5	3
Gelatin	None	5	3
Fetal bovine serum	None	6	2
None	Collagen	6	3
None	Fibronectin	6	3

Table II. Effects of pH and Dissolved Oxygen on T-PA production

Culture conditions		Period of t-PA production	Average t-PA activity	Average t-PA productivity	Average cell density[a]
pH	DO (ppm)	(day)	(IU/ml/day)	(IU/10^6 cells/day)	(cells/ml)
7.4-7.0[b]	4-0[b]	30	56	33	1.7 X 10^6
7.4	4-0[b]	28	154	100	1.5 X 10^6
7.7	4-0[b]	10	71	65	1.1 X 10^6
7.4-7.0[b]	4	30	160	44	3.6 X 10^6
7.4	4	38	836	120	7.0 X 10^6

[a] The average value at the each medium replacement.
[b] The average values of the fresh (left) and harvested (right) media.

t-PA productivity, although at a high pH of 7.7 the cells had a tendency to detach themselves from microcarriers. The t-PA activity secreted into the medium was the highest at a constant pH of 7.4. On the other hand, maintaining only DO at a constant value did not enhance the t-PA productivity. However, we found that a culture at constant DO of 4 ppm led to an increase of cell density and consequently enhanced the t-PA activity secreted into the medium. The increase of cell density on microcarriers is probably due to the decrease of cell size, and thereby a large number of cells attached to microcarriers. When the cells were cultured at a constant DO of more than 4 ppm, the same results were obtained, although the excess oxygen is reported to be detrimental to the growth of many cell types in culture.[23] The regulation of DO at a value less than 4 ppm led to a decrease of cell density (data not shown). The supply of oxygen through the micro-pores of a teflon tube steeped in the production medium was important, because the amount of oxygen supplied from the upper surface of the medium was too small to satisfy the demand of the human cells at high density. The cells derived from muscle may require large amounts of oxygen for the synthesis of high energy compounds. By regulating both factors (pH 7.4 and DO, 4 ppm), the cell density was further increased but the t-PA productivity was almost identical to that under the regulation of only pH at 7.4. These results suggest that the t-PA productivity and the cell density depend on pH and DO of the medium, respectively, and that the effect of DO on the cell density is enhanced by the regulation of pH. Under these conditions, the t-PA activity (836 IU/ml/day) corresponds to 280 times that of the initial experiment described earlier, while the t-PA productivity was almost constant over the period of t-PA production. The maximum cell density was 1.2 X 10^7 cells/ml on the 23th day, and then decreased gradually. Since the number of microcarriers per gram of cytodex 1 was 6.8 X 10^6, 575 cells attached to one microcarrier on the average. In the absence of beef extract, the cells rapidly detached themselves from microcarriers irrespective of the regulation of pH and DO.

Thus, the t-PA productivity by microcarrier culture was greatly enhanced by the culture conditions suitable for the cells.

Enzyme purification

The enzyme purified from the culture broth (60 l, 48 X 10^6 IU) according to the methods of Rijken and Collen[3] showed two protein bands upon SDS-gel electrophoresis (Fig. 2), suggesting that the purified enzyme contains two variants with different carbohydrate contents as well as the enzyme from melanoma cells.[7] The each variant was isolated by lysine-Sepharose chromatography as described above, and also subjected to SDS-gel electrophoresis (Fig. 2).

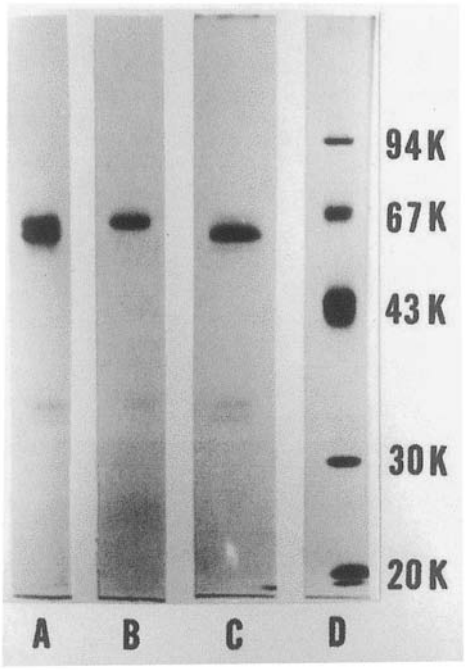

Fig. 2. SDS-Polyacrylamide Gel Electrophoresis of Purified T-PA (A) and Its Variants (B, C). The electrophoresis was carried out in 12 % acrylamide gels. Sample load was 10μg per gel. Standard proteins (D) were phosphorylase b, bovine serum albumin, egg albumin, carbonic anhydrase, and trypsin inhibitor, from top to bottom.

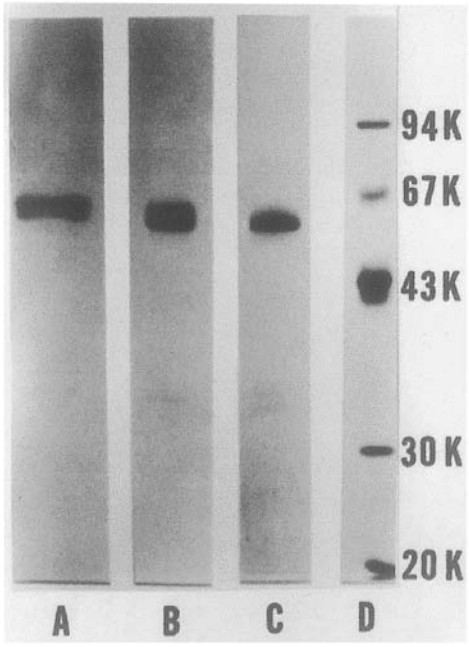

Fig. 3. SDS-Polyacrylamide Gel Electrophoresis of Native T-PA (A), T-PA treated with Endo-H (B) and Deglycosylated T-PA Isolated by Lysine-Sepharose Chromatography (C). The electrophoresis was carried out in 12 % acrylamide gels. Sample load was 10μg per gel. Standard proteins (D) were phosphorylase b, bovine serum albumin, egg albumin, carbonic anhydrase, and trypsin inhibitor, from top to bottom.

The molecular weights of the enzymes were approximately 65,000 and 63,000. The carbohydrate contents of these variants were 8.5 and 5.6 %, respectively, suggesting that the molecular weight of the protein moiety of the enzyme is calculated as about 59,500. The carbohydrate contents were different from those (6.9 and 6.7 %) reported by Randy *et al.*[12] The difference is probably due to the purity of the enzymes or the different cells used. The specific activities of the two variants from the human cells were almost identical (about 400,000 IU/mg of protein).

Preparation of carbohydrate-depleted t-PA

Enzymatically deglycosylated t-PA was prepared by digestion of purified t-PA (mixture of two variants) with Endo-H. Digestion was done for 6 h, because the digestion for a longer period (*e.g.*, 8 h) under the same conditions yield a similar result, and that for a shorter period (*e.g.*, 4 h) was insufficient to remove carbohydrate side chains of t-PA as judged from SDS-gel electrophoresis (data not shown). The digested products of t-PA were subjected to SDS-gel electrophoresis. As shown in Fig. 3, t-PA treated with Endo-H migrated as a doublet with molecular weights of 60,000 and 62,000. Since native t-PA had the molecular weight of about 63,000 and 65,000, Endo-H cleaved carbohydrate side chains with a average molecular weight of 3000 from a native t-PA molecule. The appearance of two types of t-PA still after Endo-H digestion suggests that the identical carbohydrate side chains are removed from each variant of t-PA or the variants have different sensitivities to Endo-H. To isolate deglycosylated t-PA, the reaction mixture was subjected to Con A-Sepharose chromatography. Deglycosylated t-PA obtained as the unadsorbed fraction with a yield of 46 % had the molecular weight of 60,000 (Fig. 3) and a specific activity of 390,000 IU/mg of protein, which is almost identical to that of the native enzyme. The t-PA with a molecular weight of 62,000 was bound to Con A-Sepharose and eluted with 0.4 M methyl α-D-mannopyranoside, indicating that the 62K t-PA still contains high mannose type of carbohydrate side chains. Both the t-PAs (60K and 62K t-PA) were proteins containing 0.8 and 4.0 % carbohydrate, respectively.

To prepare completely nonglycosylated t-PA, the human cells were treated with TM (*N*-glycosylation inhibitor). TM was used at the concentration of 10 μg/ml, because the treatment at higher concentration (e.g. 20 μg/ml) under the same conditions resulted in the rapid detachment of the cells from microcarriers within 2 days, and that at lower concentration (*e.g.*, 5 μg/ml) was ineffective to inhibit the glycosylation of t-PA (date not shown). The human cells treated with TM at concentration of 10 μg/ml also detached themselves from microcarriers gradually within 5 days. The average

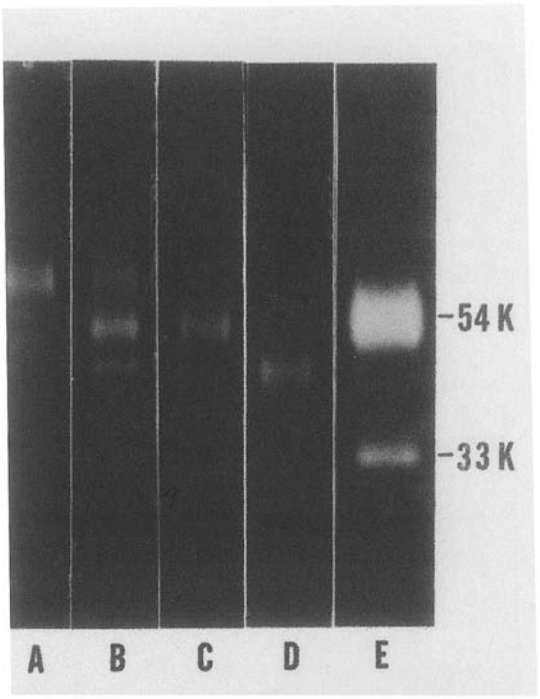

Fig. 4. Fibrin Zymography of Native and Nonglycosylated T-PA. The conditioned media over the human cells in the absence and presence of TM were shown in lanes A and B, respectively. The nonglycosylated t-PAs isolated by lysine-Sepharose chromatography were shown in lanes C and D, respectively. Lane E was HMW and LMW-UK as protein markers of the indicated molecular weights.

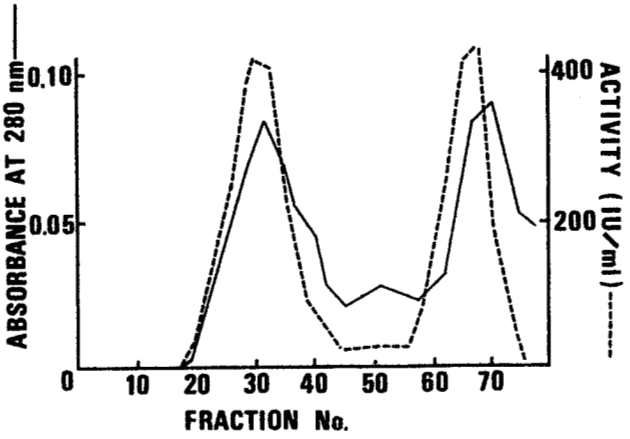

Fig. 5. Lysine-Sepharose Chromatography of Nonglycosylated T-PA Obtained from TM-treated KW Cells.
The protein concentration (—) was measured by adsorption at 280 nm, and the t-PA activity (- -) was assayed on fibrin gel plates.

Table III. Fibrin-Sepharose Chromatography of Several T-PAs

T-PA	Carbohydrate content (%)	Activity (%) eluted from the resin with	
		buffer 1[a]	buffer 2[b]
46K t-PA	0	100	0
52K t-PA	0	2	98
60K t-PA	0.8	35	65
63K t-PA	5.6	39	61
65K t-PA	8.5	65	35

[a] The equilibration buffer supplemented with 0.1 M lysine.

[b] Buffer 1 supplemented with 1 M KSCN.

activity of t-PA secreted in the medium was about 70 IU/ml which corresponds to about 8 % of that under the same conditions without TM (Table II). The decrease of activity is probably due to inhibition of the secretion of t-PA because the t-PA activity (6 IU/10^6 cells) of intracellular fractions obtained by ultrasonic oscillation (20 KHz, at 0 °C for 1 min) of the cells after treatment with TM was higher than that (1 IU/10^6 cells) before treatment with TM. The harvested medium was subjected to analysis by fibrin zymography (Fig. 4). The molecular weights of t-PAs in the medium were approximately 52,000 and 46,000 except that of native t-PA. To remove native glycosylated t-PA, the medium was subjected to Con A-Sepharose chromatography. Most of the fibrinolytic activities (80 %) of the medium did not adsorbed to the resin. The unadsorbed fraction was further subjected to lysine-Sepharose chromatography. As shown in Fig. 5, the activity was eluted as two peaks at concentration of about 30 and 70mM arginine, and both peaks were pooled and analyzed by fibrin zymography. The early-eluting peak fraction was found to contain t-PA with a molecular weight of 46,000, and the late-eluting peak contained the t-PA with a MW of 52,000 (Fig. 4). Carbohydrate was not detected in either fraction, indicating that t-PA from the human cells undergoes *N*-glucosylation, but not *O*-glycosylation. Since the molecular weight of the protein moiety of the native enzyme was about 59,500 as determined above, the two t-PAs obtained from the human cells treated with TM may lack not only sugar moieties, but also may have lost peptide fragments with molecular weights about 7500 or 13,500, probably because of proteolytic degradation. The cleavage of a protein moiety is not reported in similar experiments with rCHO cells[8] and melanoma cells.[9] The difference is probably due to that of the cells used in the experiment.

Binding of several t-PAs to fibrin

The binding of native glycosylated and deglycosylated t-PAs to fibrin was compared by fibrin-Sepharose chromatography. Most activities of each t-PA applied to the column were found to bind to the affinity gels. After the each gel was washed with equilibration buffer, stepwise elution was carried out with the same buffer containing 0.1 M lysine (buffer 1) and then with the same buffer containing 0.1 M lysine and 1 M KSCN (buffer 2). The fibrinolytic activities in the two eluates were summarized in Table III. The t-PA eluted with buffer 2, but not buffer 1, shows stronger binding to fibrin than that eluted with buffer 1. The activity recovered in buffer 2 increased with the decrease of the carbohydrate content of t-PA except for 46K t-PA. The 52K t-PA showed the strongest binding to fibrin, although the nonglycosylated enzyme lacks a peptide fragment as described above. These results suggest that carbohydrate side chains of t-PA modulate the binding strength of the enzyme to fibrin, and that TM-treatment of the human cells yields a new t-PA with a strong affinity of fibrin.

The present results demonstrated the role of carbohydrate moiety of t-PA obtained from a cell line established from human normal uterus. The native t-PA synthesized in the human body may have different carbohydrate side chains from that of the t-PA used in this report, and also from that of the t-PA obtained by culture of normal human cells. The true role and structure of carbohydrate moiety of native t-PA awaits further investigation.

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