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Some chemical properties of tissue plasminogen activator purified from paranasal mucous membrane

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SUMMARY

Plasminogen activator (PA) was purified from an acetone powder preparation of paranasal mucous membrane with chronic sinusitis, and some chemical properties of the purified PA were investigated in this paper. Zn-imminodiacetate affinity chromatography, lysine sepharose affinity chromatography and ultrafiltration for concentrating a PA fraction were consecutively performed to purify the PA from the acetone powder preparation. Finally, gel filtration was performed using Sephacryl S-200 in order to estimate the molecular weight of the purified PA. The purified PA in this experiment showed a stronger affinity to fibrin than urokinase did. The molecular weight of the purified PA was estimated to be 65,000 to 70,000 daltons as determined by Sephacryl S-200 gel filtration. The Km of the purified PA was 0.11 mM. From these results, it is apparent that the PA purified from an acetone powder preparation of paranasal mucous membrane belongs to the class of tissue type plasminogen activators (t-PA)

INTRODUCTION

It is known that large amounts of plasminogen activator are contained in tissue extracts of paranasal mucous membrane with chronic sinusitis (Sasaki et al., 1959; Kosugi et al., 1982).

Plasminogen activator in general is classified into two types, that is, urokinase-type plasminogen activator and tissue-type plasminogen activator according to its chemical properties (Danø et al., 1985). Generally speaking, it has been found that tissue-type plasminogen activator (t-PA) is more effective as a thrombolytic agent than the urokinase-type plasminogen activator (u-PA) (Matsuo, 1981). In this study we attempted to isolate and purify plasminogen activator from an acetone powder preparation of paranasal mucous membrane.

Furthermore, some of the chemical properties of the purified plasminogen activator were studied in order to clarify the type of PA.

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MATERIALS AND METHODS

Paranasal mucous membrane was surgically removed from twenty patients with chronic sinusitis. Acetone powder preparations of paranasal mucous membrane were made in accordance with a previously reported method (Kosugi et al., 1982). Fifty ml of 2M KCl was added to 1.0 g of acetone powder and stirred in a cold room (4°C) for overnight. After stirring the homogenate was centrifuged at 10,000 r.p.m. for twenty minutes. The resulting supernatant was used as the starting material, i.e., the crude extract in the subsequent investigations. Affinity chromatography using Zn-imminodiacetate sepharose was performed following the method of Porath (1975) and Hubert (1980).
Lysine sepharose affinity chromatography was performed according to Deutsch and Mertz's method (1970). Fibrinolytic activity was ascertained using the fibrin plate method (Astrup and Müllertz, 1952). Amidase activity was determined using synthetic substrate, S-2288 (Matsuo et al., 1983).

RESULTS

1. *Purification of t-PA from paranasal mucous membrane*

The purification procedure and the yield at each step are summarized in Table 1. An approximately 107-fold increase in specific activity was attained with an overall yield of 52.3%.

Table 1. Summary of the purification process of t-PA from paranasal mucous membrane. Zn-sepharose in table indicates Zn-imminodiacetete sepharose affinity chromatography. Lysine sepharose in table indicates lysine-sepharose affinity chromatography.

	total protein (A_{280})	total activity mm^2	specific activity (mm^2/A_{280})	purity	recovery (%)
crude extract	235	$1.830 x 10^3$	$7.79 x 10^3$	1.00	100
Zn-sepharose	40.3	$2.410 x 10^3$	$59.80 x 10^3$	7.68	132
lys.-sepharose	1.65	$1.370 x 10^3$	$830.0 x 10^3$	107	74.9
ultrafiltration	1.74	$957 x 10^3$	$550.0 x 10^3$	70.6	52.3

2. *Determination of molecular weight*

As shown in Figure 1, the purified t-PA from the paranasal mucous membrane formed one peak. Furthermore, using known marker proteins, the eluted volume of the purified t-PA was found to be near that of BSA. The molecular weight of the purified t-PA from the paranasal mucous membrane was estimated to be about 65,000–70,000 daltons using gel filtration with Sephacryl S-200.

3. *Amidase activity of purified t-PA from paranasal mucous membrane*

The amidase activity of the t-PA was determined using synthetic substrate

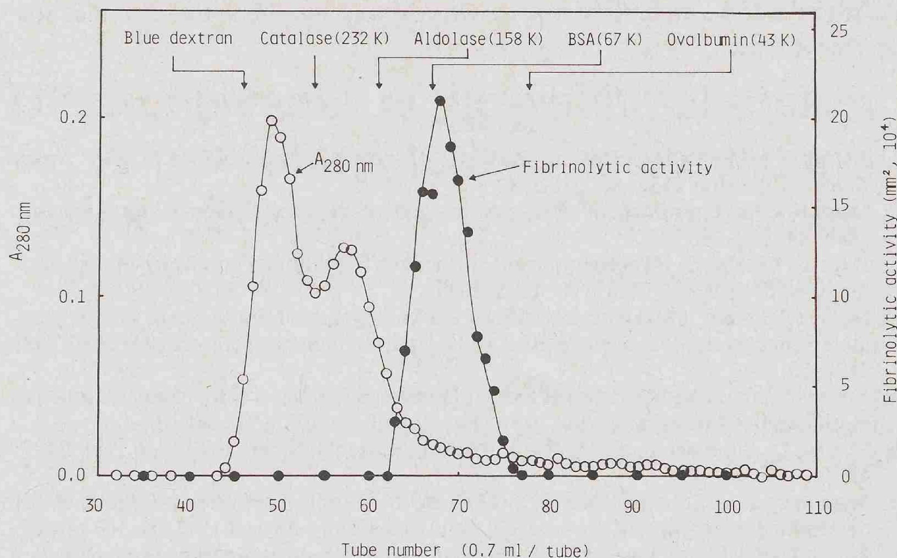
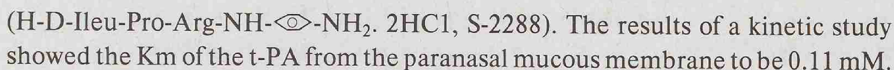


Figure 1. Chromatography of t-PA from paranasal mucous membrane on a Sephacryl S-200 column. Blue dextran, catalase, aldolase, BSA (Bovine Serum Albumin) and ovalbumin were used as known marker proteins.


(H-D-Ileu-Pro-Arg-NH-<◎>-NH_2. 2HC1, S-2288). The results of a kinetic study showed the Km of the t-PA from the paranasal mucous membrane to be 0.11 mM.

4. *Effect of pH on the stability of purified t-PA from paranasal mucous membrane*
The fibrinolytic activity of the plasminogen activator was stronger under alkaline conditions than under acidic ones.

DISCUSSION

In this study, we succeeded in isolating and purifying plasminogen activator from extracts of acetone powder preparation of paranasal mucous membrane with chronic sinusitis. Results of studies on some of the chemical properties of the purified plasminogen activator showed that the purified plasminogen activator belongs to the class of tissue-type plasminogen activators. First of all, the molecular weight of the purified plasminogen activator is about 65,000–70,000 daltons. Secondly this purified plasminogen activator has a strong affinity for fibrin clots. Thirdly, from a kinetic study using a synthetic substrate the Km was found to be 0.11 mM, $1.1 / 10^{-4}$ mol/l.

Based upon the above mentioned results we confirmed that the purified plasminogen activator used in this experiment was tissue-type plasminogen activator (t-PA) of the two chain variety. It is suggested that the t-PA derived from human

paranasal mucous membrane is more effective as a thrombolytic agent than the urokinase type.

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