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CHEMICAL CHARACTERIZATION OF THE PURIFIED COMPONENT OF SPECIFIC TRANSFER FACTOR IN THE LEUKOCYTE DIALYSATES FROM HSV-1 IMMUNIZED GOATS

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Summary. - The chemical characterization of the purified component responsible for HSV-1 specific transfer factor activity (PTFC) by high resolution analytical methods was performed. PTFC had a molecular weight of 6,000 dalton by the size-exclusion HPLC analysis; it showed a marked UV-absorbance spot at 254 nm and a fluorescent spot at 366 nm on the thin-layer plate by thin-layer chromatography which spots coincided at the same place of the plate. The amino acid composition and sequencing analyses showed that PTFC consisted of at least twelve different amino acids, but the amino acid sequence could not be determined. The combined results indicate that PTFC is a compound with a molecular weight of 6,000 dalton, composed of peptide and nucleotide-like material. The peptide is rich in aspartic acid and its N-terminal end may be blocked.

Key words: purified transfer factor component; herpes simplex virus type 1; chemical characterization

Introduction

During the last 30 years, crude or partially purified transfer factor (TF) preparations have been used for its chemical characterization due to the lack of highly purified sample (Foster *et al.*, 1979; Wilson *et al.*, 1981; Huang *et al.*, 1987). The results were seriously impaired by impurities contained in the preparations. The degree of purification of various TF preparations differed in different laboratories and no identical views have been reached on the chemical nature of transfer factor so far. Some research groups have attempted to determine the TF structure indirectly via enzyme inactivation assays (Burger *et al.*, 1979; Wilson *et al.*, 1982). The models assumed showed a ribonucleopeptide structure for TF, and the molecular weight of the TF from dialy-

zable leukocyte extract was estimated as 1,000-2,000 dalton (Wilson *et al.*, 1982). However, some differences between these models were observed, and the molecular weight of TF also differed according to the results of other research groups (Foster *et al.*, 1979; Huang *et al.*, 1987).

Most recently, a high purified active component of transfer factor specific for HSV-1 (PTFC) has been obtained in our laboratory by a very powerful purification procedure consisting of affinity chromatography and RP-HPLC was described (Qi *et al.*, 1992). It has become possible to understand directly the chemical nature of TF. In this paper, the characterization of PTFC was determined through highly resolution analytical methods, and the amino acid sequence of PTFC was further approached.

Materials and Methods

Analytical size-exclusion high performance liquid chromatography (Size-exclusion HPLC) (Yao and Ma, 1984). Chromatographic conditions: experiment was performed with a High performance liquid chromatograph 721 (Millipore-Waters Associates), equipped with a variable ultraviolet detector and a Model 721 system controller. A 7.5 mm × 60 cm Ultropark TSK-G2000SW column (LKB) was used and the eluate for separation on TSK-gel filtration column was 0.1 mol/l phosphate buffer at pH 6.8. Measurement was made at 214 nm in the sensitivity range of 0.05-0.2 absorption units full scale. The flow rate was 1 ml/min at room temperature.

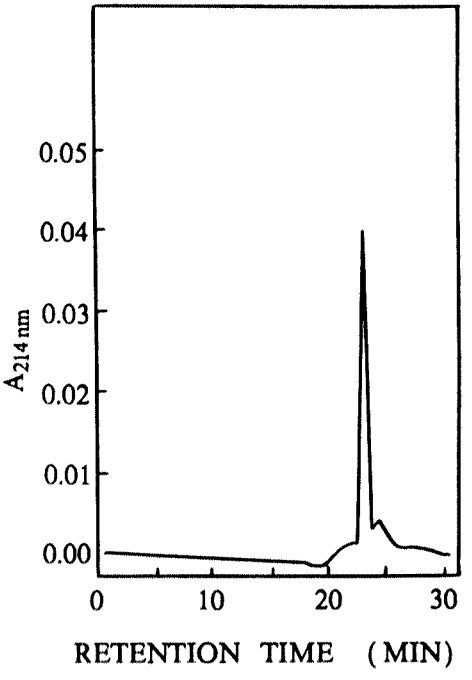

Fig. 1
Size-exclusion HPLC of PTFC on a TSK-G 2000 SW 0.75 × 60 cm column Chromatography was performed at a flow rate of 1 ml/min with phosphate buffer (0.1 mol/l, pH 6.8).

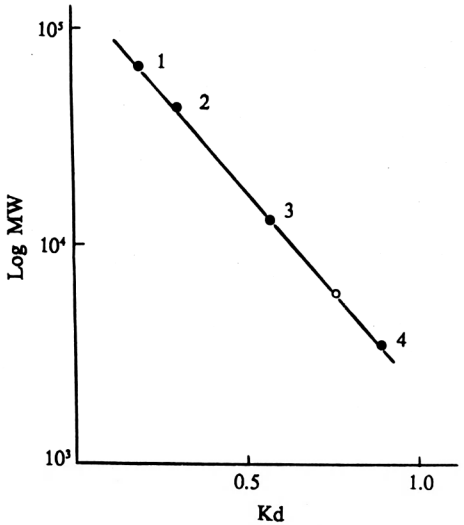

Fig. 2
Determination of molecular weight of PTFC on Size-exclusion HPLC
Four standard proteins (●): 1) Bovine serum albumin; 2) Ovalbumin; 3) Ribonuclease; 4) Insulin, B chain, and PTFC (○).

Thin-layer chromatography (Gooderham, 1984). A HF-254 thin-layer plate was used. The sample was separated by ascending thin-layer chromatography in a mixture of butan-l-ol, acetic acid, water and pyridine (15:3:10:12 vol to vol). The UV-absorbance spot of the sample on the plate was first observed at 254 nm. Then, the plate was sprayed with the fluorescamine solution and dried. The fluorescent spot was observed by means of a long-wave (366 nm) UV lamp.

Amino acid analysis. The sample was hydrolyzed in 6 mol/l HCl for 24 hr at 105 °C and analyzed in PICO-TAG amino acid analytical system (Millipore-Waters, U.S.A.) according to Bergman *et al.* (1986).

Amino acid sequence analysis. Sequencing of the sample was performed with protein sequence automatical analyzer (ABI, U.S.A.) according to the Edman degradation principle.

Results and Discussion

Molecular weight of purified TF component specific for HSV-1

The molecular weight (M.W.) of PTFC was determined with size-exclusion HPLC. The four proteins: bovine serum albumin (M.W. 66,300), ovalbumin (M.W. 43,000), ribonuclease (M.W 13,000) and insulin B chain (M.W. 3,400) were used as standard proteins. The result showed only one UV-absorbance peak at 214 nm on the HPLC chromatogram (Fig. 1), and the M.W. of the peak was 6,000 dalton (Fig. 2).

Thin-layer chromatography of purified TF component specific for HSV-1

PTFC showed a marked UV-absorbance spot at 254 nm and a fluorescent spot at 366 nm on the thin-layer plate and both spots coincided at the same position on the TLC plate (Fig. 3). This suggests that PTFC is composed of a peptide and a nucleotide-like material. However, further evidence on the presence of the peptide should be obtained as the fluorescamine reagent could

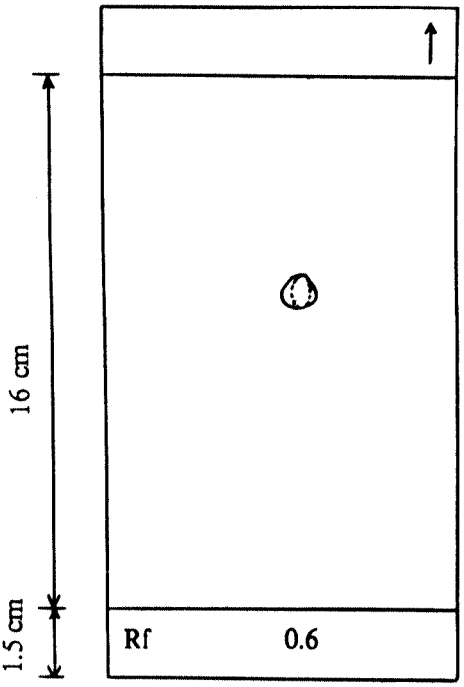

Fig. 3
Thin-layer chromatography of PTFC on 10 × 20 cm silica gel HF 254 plate
A 254 nm absorbent spot of PTFC (——) and a 366 nm fluorescent spot of PTFC (---) were overlapping (RF = 0.6).

combine with primary amines of both the peptide and free amino acids to elicit fluorescence at 366 nm.

Amino acid composition and sequencing of the purified TF component specific for HSV-1

We aimed to analyze amino acid composition of the PTFC peptide. No free amino acid was found in PTFC sample. However, at least twelve different

Table 1. Amino acid composition of PTFC

Amino acids	Retention time (min)	Peak area (%)
Asp	1.89	44.2
His	5.14	16.2
Arg/Thr	5.91	6.0
Val/Pro	6.32	7.8
Tyr	8.14	4.7
Met	9.14	1.6
Ile/Leu	9.83	5.6
Phe	10.83	2.5
Lys	11.38	11.4

amino acids were identified in the hydrolyzed PTFC sample when the peptide-bonds were hydrolyzed by 6 mol/l HCl. Asparatic acid (Asp) was the most frequent among these amino acids (Table 1).

In further experiment, we attempted to approach the primary structure of the peptide in PTFC because the peptide moiety of TF was the important component responsible for the antigen-specificity of TF (Chase, 1983). However, no N-terminal amino acid could be detected by sequencing analysis. Therefore, it was inferred that the N-terminal end of the peptide in PTFC might be blocked.

A block of the N-terminal end is common in biologically active peptides and natural proteins. However, it seems not easy to resolve the block of N-terminal end of the peptide part due to very small amounts of the purified TF sample available. Therefore, an additional powerful technique which is not limited by the N-terminal end block should be used for the determination of the peptide sequence, such as mass spectrophic analysis (Biemann, 1986).

In conclusion, our data presented here demonstrate that the purified TF component specific for HSV-1 is a compound with molecular weight of 6,000 dalton, composed of a peptide and nucleotide-like material. The peptide part is rich in asparatic acid and its N-terminal end may be blocked.

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