PSEUDORABIES VIRUS GROWTH FACTOR CAN BE RESOLVED INTO TWO ACTIVE COMPONENTS

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Summary. – Pseudorabies virus (PRV) growth factor (PRGF) which induces a transformed phenotype in normal MK-2 cells and represses the transformed phenotype of Hela cells was partially purified and resolved into two components (M_r<300 and <180). Each of the PRGF components retained the transforming activity of the original factor in MK-2 cells but lost its transformation-repressing activity in Hela cells. The latter activity of PRGF could be reconstituted by simultaneous application of its two components. Two monoclonal antibodies against gII glycoprotein of PRV were able to neutralize both PRGF activities, thus supporting the previously suggested hypothesis that the PRV gene for glycoprotein gII might be involved in PRGF synthesis.

Key words: pseudorabies virus growth factor; purification; discontinuous recyclization chromatography; two-component character

A putative growth factor, probably encoded by PRV was detected in mammalian cells transformed by PRV as well as in cells infected with PRV and cultivated under conditions non-permissive for virus replication. The PRGF was shown to have two different effects on cultured cells *in vitro*. Normal, non-transformed cells cultivated in the presence of PRGF acquire the phenotype of transformed cells ("criss-cross" pattern of growth, formation of colonies in soft agar), while the phenotype of transformed cells exposed to PRGF changes to the normal one (Golais *et al.*, 1990; Golais *et al.*, 1992*a*). Similar growth factors (HSGF-1, HSGF-2) were shown to be produced by cells infected with human herpes simplex viruses type 1 and 2 (HSV-1, HSV-2) (Golais *et al.*, 1992*a*; Golais *et al.*, 1992*b*). Neutralization of HSGF-2 activity with monoclonal antibodies (MoAbs) against the HSV-2 glycoprotein B, and the properties of intratypic recombinants in the HSV-1 glycoprotein B gene implied a role of the glycoprotein B gene or its product in the formation of HSGF-1 and HSGF-2 (Golais *et al.*, 1992*a*; Golais *et al.*, 1992*b*). The chemical nature of the PRGF as well as its mode of action are at present unclear.

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For further biological and chemical characterization PRGF underwent a gentle but effective purification by discontinuous recycling chromatography (Morávek 1971). In the present paper we describe an efficient purification of PRGF and its resolution into two different components.

Monkey kidney (MK-2) cells grown in Eagle' s basal medium with 5% heated bovine serum were infected with TOP strain of PRV at input multiplicity of 1 PFU/cell. The medium from infected cultures was harvested 3 days p.i., acidified to pH 2.5, kept for 3 days at 4 °C to inactivate the residual virus (Golais *et al.*, 1990) and then adjusted to pH 7.2. The medium (4 liters) was then concentrated by vacuum evaporation (40 – 50 °C) to a volume of 400 ml ("concentrated medium"). It was divided into 50 ml aliquots which were applied onto a Sephadex G-15 column (5 × 80 cm). During elution by deionized water 100 ml fractions were collected (Fig. 1). The active fractions from 8 separations were collected and concentrated by vacuum evaporation to 1/20 of original volume. In the next step, the discontinuous recycling chromatography, these concentrates were consecutively applied on the same Sephadex G-15 column in the order in which they were eluted in the first step. After this rechromatography the PRGF cell transforming activity appeared in two peaks: $PRGF_A$ fraction (total volume 300 ml) and $PRGF_B$ fraction (280 ml) (Fig. 2). Each fraction was concentrated in vacuum to 3 ml, clarified by centri-

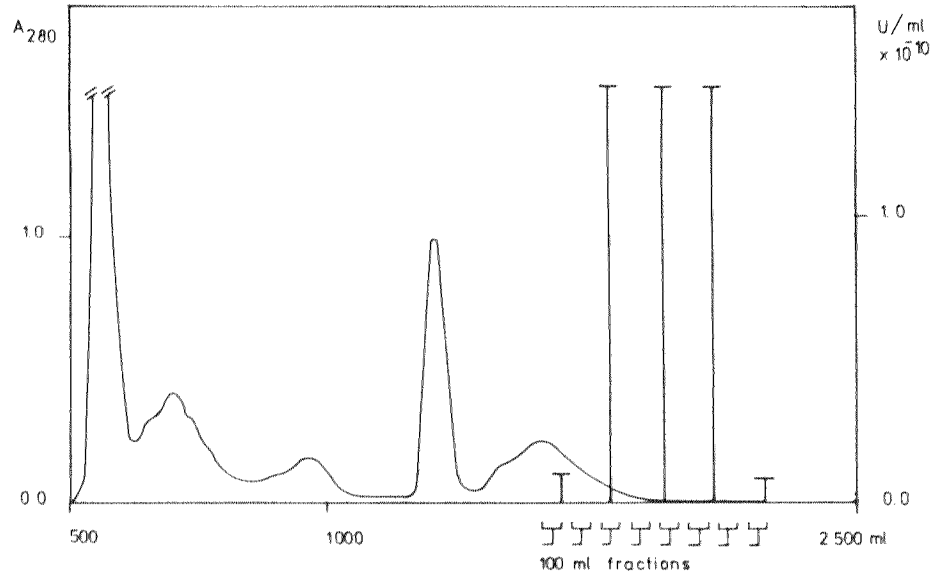

Fig. 1
Chromatography of PRGF on Sephadex G-15 column
Abscissa: eluate volume (ml); left ordinate (continuous line): A_{280}; right ordinate (bars): TA (U/ml).

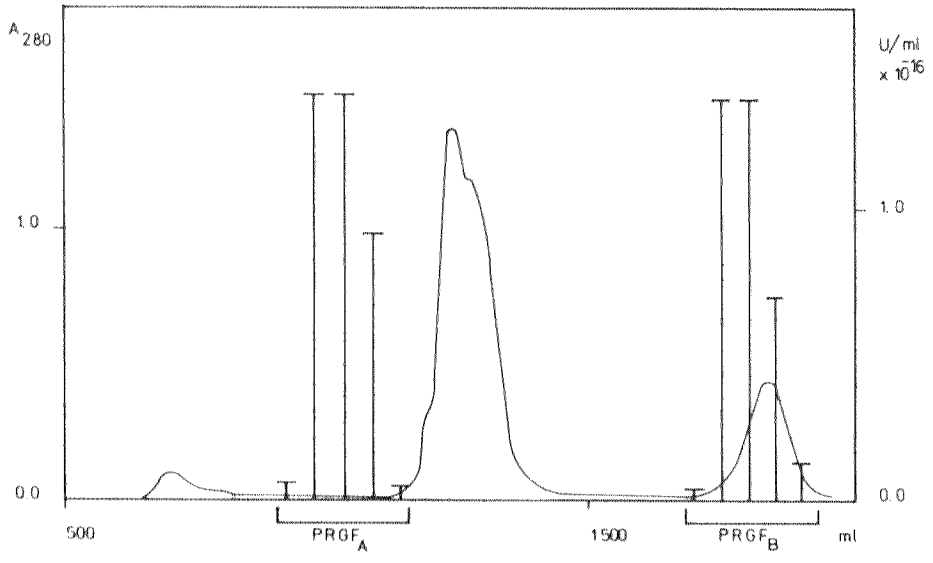

Fig. 2
Recycling chromatography of PRGF on Sephadex G-15 column
Abscissa: eluate volume (ml); left ordinate (continuous line): A_{280}; right ordinate (bars): TA (U/ml).

fugation at 15,000 × g for 5 mins and applied to a Bio-Gel P2 column (2 × 38 cm) (Fig. 3). The active purified fractions $PRGF_A$ and $PRGF_B$ recovered after eluting the column with water were lyophilized and 478 mg of $PRGF_A$ or 4 mg of $PRGF_B$ were obtained.

Fractions obtained during purification were tested for the transforming activity (TA) (Golais *et al.*, 1992*a*) in normal cells (MK-2), the ability to repress the transformed phenotypes of Hela cells, i.e. the transformation-repressing activ-

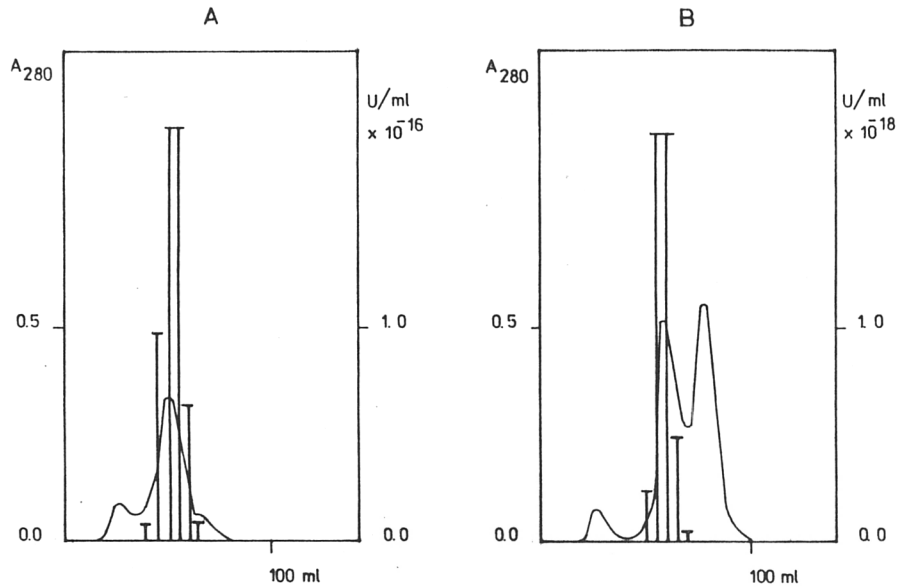

Fig. 3
Chromatography of $PRGF_A$ (A) and $PRGF_B$ (B) components on Bio-gel P2 column
Abcsissa: eluate volume (ml); left ordinate (continuous line): A_{280}; right ordinate (bars): TA (U/ml).

Table 1. The transforming and transformation-repressing activities (TA, TRA) of PRGF and its components $PRGF_A$ and $PRGF_B$, and their inhibition by MoAb directed against gII of PRV

Material	TA (U/ml)		TR (U/ml)	
	Control[a]	MoAb36	Control[a]	MoAb38
Concentrated medium	1×10^5	$<1\times 10^1$	2.5×10^4	1×10^1
$PRGF_A$ fraction (Sephadex G-15)	1×10^7	$<1\times 10^1$	$<1\times 10^1$	nd
$PRGF_B$ fraction (Sephadex G-15)	1×10^7	$<1\times 10^1$	$<1\times 10^1$	nd
$PRGF_A + PRGF_B$ fractions (1:1)	1×10^7	$<1\times 10^1$	2.5×10^6	2.1×10^6

[a]Tested samples were diluted to given titers before testing with MoAb.
nd – not done.

ity (TRA) and the neutralization of both activities with MoAbs (MoAb 36 and MoAb 38) (Qvist *et al.*, 1989) directed against the PRV glycoprotein gII. The assays of TA, TRA as well as the neutralization method with MoAbs were described previously (Golais *et al.*, 1990; Golais *et al.*, 1992*a*).

One TA or TRA unit (U) represents the endpoint dilution of PRGF sample causing transforming or transformation-repressing effect in 50% of cell cultures. Transformed phenotype ("criss-cross" cell morphology, anchorage-independent growth) of PRGF-treated MK-2 cells was acquired without any dependence on PRGF concentration (TA 10^{-3}-10^{-9} U/ml), and similarly, the PRGF concentration had no influence on repression of transformed phenotype of Hela cells. MoAbs 36 and 38 in the form of ascitic fluid were obtained from Veterinary Serum Laboratory, Copenhagen, Denmark (Qvist *et al.*, 1989). These MoAbs diluted 1:100 (Golais *et al.*, 1992*a*) completely neutralized both TA and TRA of PRGF. These findings considerably facilitated the testing or both activities of PRGF and its two components during purification (see Table 1).

Both TA and TRA of PRGF were retained after the first step of purification and were neutralized by the MoAb 36 or 38 (Table 1). Both $PRGF_A$ and $PRGF_B$ possessed TA against MK-2 cells that could be inhibited by MoAb 36 or 38. The separated PRGF components lost the TRA, but it was restored when they were combined 1:1 (v/v) and used simultaneously. However, the reconstituted TRA was not neutralized by the MoAbs employed. It is possible, that a factor essential for the inhibition of this PRGF effect by the antibodies was removed during purification.

Coomasie brilliant blue and silver-staining of SDS-PAGE gels of purified and lyophilized $PRGF_A$ and $PRGF_B$ components after electrophoresis (Laemli, 1971) did not reveal any protein band in the preparations (data not shown). As detected by determining the TA of the extracts of gel slices after SDS-PAGE of $PRGF_A$ and $PRGF_B$, both components migrated together with the electrophoretic front. The estimation of M_r of purified and lyophilized $PRGF_A$

and $PRGF_B$ by gel filtration on the FPLC Superose-12 column also showed their low-molecular weight character. The retention time of $PRGF_A$ was higher than that of glutathion (M_r 307), and that of $PRGF_B$ was higher than that of tyrosine (M_r 181). Thus it can be concluded that M_r of $PRGF_A$ and $PRGF_B$ are lower than 300 and 180, respectively. Except of the growth factor produced by cells transformed by adenovirus 12 (M_r<1000) which may be a lipid or oligopeptide (Tsuji *et al.*, 1992), all the known virus-related growth factors have higher M_r and are predominantly proteins. The two components of PRGF thus belong to the smallest virus-associated growth factors described so far.

The attempts to characterize chemically the nature of $PRGF_A$ and $PRGF_B$ are at present in progress in our laboratory.

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