SERUM FACTORS STIMULATING DNA SYNTHESIS IN THE ISOLATED NUCLEAR SYSTEM FROM RAT LIVER

Kiyokazu Morioka, Hiraku Shimada and Hiroshi Terayama

Zoological Institute, Faculty of Science, University of Tokyo, Tokyo, Japan

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SUMMARY ^{3}H-TTP incorporation into DNA by the isolated rat liver nuclei was stimulated by the rat serum in proportion to its concentration. Dialysis and gel-filtration of the serum indicated the presence of two factors: one is low-molecular and another is high-molecular. The high-molecular factor is thermolabile while the low-molecular one is thermostable. The latter is resistant to pronase-treatment and can not be adsorbed on charcoal. The sera from normal and partially hepatectomized rats showed similar stimulatory effect.

INTRODUCTION

Although the *in vivo* effects of serum or its components on the DNA synthesis or mitosis of liver cells, especially with respect to the liver regeneration, have been investigated rather extensively (1-7), we still know little about the real mechanisms of the homeostatic control of liver cell proliferation. As to the *in vitro* effects of serum on the cellular proliferation several papers (8-10) have been published, showing the dose-dependent stimulatory effect of serum on cultured cell proliferation. Todaro *et al.* (11) have reported a macromolecular serum factor initiating cell division in a contact-inhibited mammalian cell line. Recently Paul *et al.* (12) have shown that primary cultures of differentiated liver cells from fetal rats respond to thoroughly dialyzed serum by an increased uptake of ^{3}H-thymidine and ^{3}H-leucine. Salas and Green (13), and Fox and Pardee (14) have indicated that the synthetic pattern of DNA-binding proteins, which might be involved in controlling DNA synthesis, is altered by the

addition of serum. Yet no paper has been published so far concerning the direct action of serum on the DNA synthesis in isolated nuclei. In this paper we wish to report our recent findings that the serum from normal or partially hepatectomized rats can stimulate ^{3}H-TTP incorporation into acid insoluble material in the isolated nuclear system prepared from normal or regenerating livers of rats and that there are at least two stimulatory factors in the sera.

MATERIALS AND METHODS

The nuclei were prepared from the normal or regenerating liver of male, Wistar rats weighing about 100g according to the method of Lynch et al (15) and were suspended in 2.5 ml of 0.3 M sucrose-4 mM $MgCl_2$ solution by means of a glass homogenizer with a loose fitting rubber pestle (3 up-and-down strokes by hand).

The reaction mixture for the DNA synthesis contained 47 mM Tris-HCl buffer (pH 7.4), 6 mM $MgCl_2$, 15 mM KCl, 7.7 mM 2-mercaptoethanol, 0.08 mM each of dATP, dGTP, and dCTP, 0.02 mM ^{3}H-TTP (Methyl-^{3}H-TTP, Radiochemical Centre, England: original sample having the specific activity of 13.6 Ci/m mole was diluted with cold TTP to get the specific activity of 280 mCi/m mole), 1.6 mM ATP and the isolated nuclei equivalent to 30-80 μg DNA in a final volume of 180 μl. The whole reaction mixture was incubated at 37° for a certain period of time and the reaction was stopped by adding 3 ml of ice-cold 5 % CCl_3COOH. Bovine serum albumin of about 2 mg was added to each tube to help complete precipitation of DNA. The precipitate was once washed with cold 5 % CCl_3COOH, washed 3 times by repeated dissolution in 0.3 ml 1N NaOH and reprecipitation with 4 ml of 10 % CCl_3COOH, and finally suspended in 1 ml of 5 % $HClO_4$. The suspension

was then heated at 100° for 10 min, and cooled immediately. After centrifugation the supernatant (about 1 ml) was subjected to radioactivity measurement in a liquid scintillation spectrophotometer (the toluene-triton X-PPO-POPOP system was used as a scintillation fluid).

The serum was separated by centrifuging the coagulated blood of male Wistar rats at 15,000 x g for 10 min, and stored at -20° until use. Dialysis of serum was performed in a Visking tube at 5° against 20 mM Tris-HCl buffer, pH 7.6 - 20 mM NaCl. Dialysis against an equal volume of the Tris-NaCl solution (1:1 dialysis) was performed overnight (18 h), while in case of complete dialysis (1:∞dialysis) the outer fluid of a large volume was replaced 3 times during 48-h dialysis. Partial hepatectomy of rats was performed according to Higgins and Anderson (16), removing the left lateral and median lobes. DNA was estimated by a little modified method of Schmidt-Thannhauser-Schneider (17) combined with the conventional colorimetry using diphenylamine (18) and calf thymus DNA as standard.

RESULTS AND DISCUSSIONS

As illustrated in Fig. 1A, the incorporation of ^{3}H-TTP into acid-precipitable DNA in the isolated normal liver nuclei increases almost linearly with time during 30 min of incubation. Addition of sera prepared from either normal rats or partially hepatectomized (20 h after operation) rats to the incubation mixture at the concentration of 28 % resulted in the marked (about 7-fold) stimulation of the ^{3}H-TTP incorporation. As shown in Fig. 1B, the ^{3}H-TTP incorporation of the isolated nuclei prepared from the regenerating liver proceeds with a much greater rate at the beginning of incubation as compared

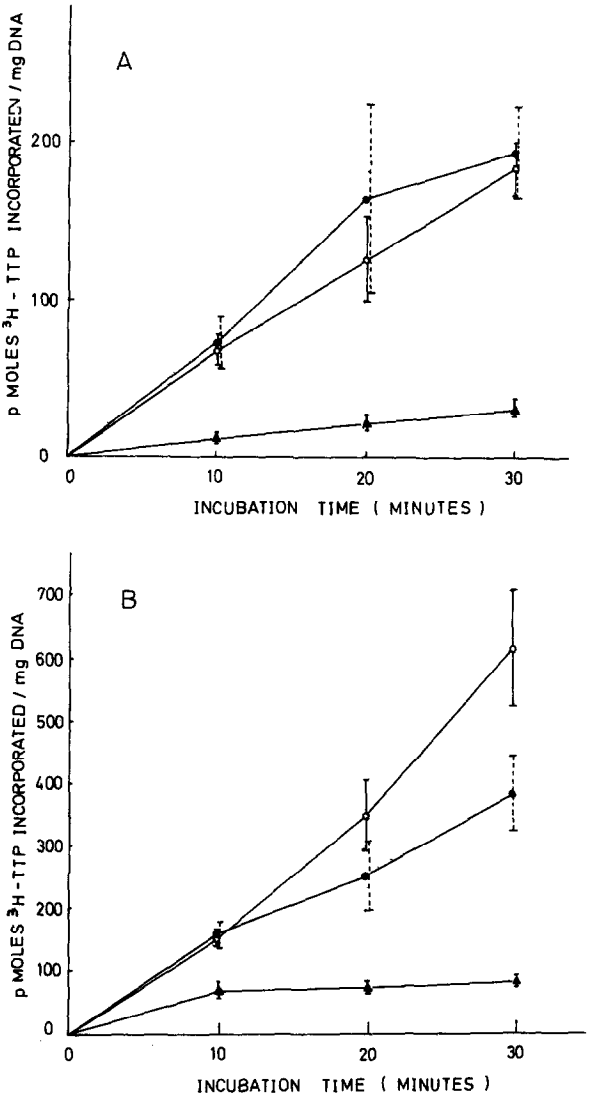

Figure 1. Effects of rat sera on the rate of DNA synthesis in the isolated nuclear system prepared from normal rat liver (Fig. 1A) and from regenerating rat liver (Fig. 1B).
▲, ○ and ● indicate the control (absence of serum), the presence of normal rat serum (28 %) and the presence of partially hepatectomized rat serum (28 %), respectively. Bars represent standard error.

with that from the normal liver but the incorporation seems to stop at 10 min of incubation. Addition of sera either from normal rats or from partially hepatectomized rats (20 h after operation) can similarly stimulate the ^{3}H-TTP incorporation into

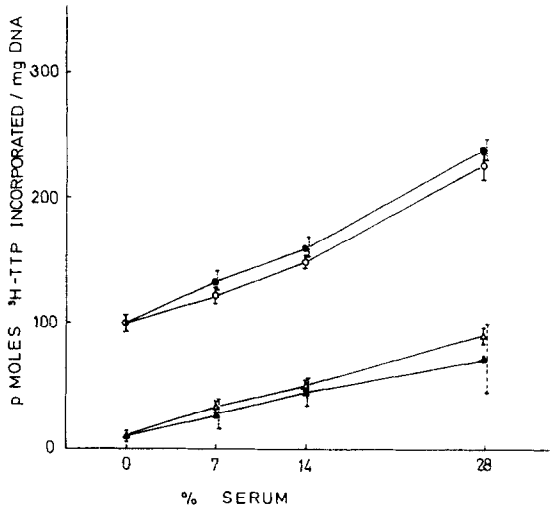

Figure 2. Concentration-dependent stimulatory effect of serum on the DNA synthesis of isolated rat liver nuclei. The isolated nuclei prepared from normal rat liver were incubated for 10 min in the presence of various concentrations of normal rat serum (Δ) or of partially hepatectomized rat serum (▲), and the isolated nuclear system prepared from regenerating rat liver were incubated for 10 min in the presence of various concentrations of normal rat serum (○) or of partially hepatectomized rat serum (●). Bars represent standard error.

DNA in the regenerating liver nuclei. It is interesting to note that the incorporating capacity of the regenerating liver nuclei is elongated or stabilized in the presence of sera.

The stimulatory effect of serum is clearly dependent on its concentration as evidenced in Fig. 2.

The normal rat serum was mixed with an equal volume of 0.9 % NaCl and aliquots of the diluted serum were treated at 80° or 100° for 5 min. The effects of the heated and unheated sera were compared on the normal rat liver nuclear system. As summarized in Table 1, almost 50 % of the activity seems to be lost by such heat treatment.

As summarized in Table 2, the inside fluid of 1:1 dialysis retains 64 % of the stimulatory activity of the whole serum,

Table 1. Effect of heat-treatment on the serum acitivity to stimulate the nuclear ^{3}H-TTP incorporation into acid-insoluble DNA.

Additive	Serum conc.(%)	^{3}H-TTP incorporated (p moles/mg DNA/10 min)	% Stimulation
None (0.9 % NaCl instead of serum)	0	11.2	0
Serum, unheated	14	49.1	338 (100)[a]
Serum, heated at 80° for 5 min	14	28.3	153 (45.2)
Serum, heated at 100° for 5 min	14	30.1	169 (50.0)

a : Relative activity

Table 2. Dialyzable and non-dialyzable factors in serum.

Exp. No.	Additive	Serum conc.(%)	^{3}H-TTP incorporated (p moles/mg DNA/10 min)	% Stimulation
I	None (deionized water instead of serum)	0	9.8	0
	Serum, undialyzed	28	138	1310(100)[a]
	Serum 1:1 dialyzate (outside)	14[b] (as to low-mol. component)	44.5	358(27)
	Serum 1:1 dialyzate (inside)	14[b] (as to low-mol. component) 28[b] (as to high-mol. component)	91.5	834(64)
	Serum 1:1 dialyzate (outside), heated at 80°, 5 min	14[b] (as to low-mol. component)	46.8	377(29)
	Serum 1:∞ dialyzate (inside)	28[b] (as to high-mol. component)	68.4	587(45)
II	None (deionized water instead of serum)	0	7.8	0
	1/2 Serum 1:∞ dialyzate (inside)	14[b] (as to high-mol. component)	31.2	300
	1/2 Serum 1:∞ dialyzate (inside) heated at 100°, 5 min	14[b] (as to high-mol. component)	7.8	0
	1/2 Serum, heated at 100°, 5 min	14	31.2	300

a : Relative activity

b : The volume was assumed to remain unchanged during dialysis.

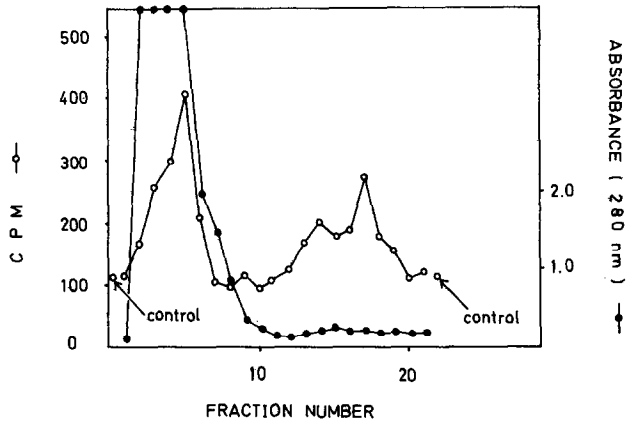

Figure 3. Separation of low- and high-molecular serum factors by gel-filtration. A 1 x 16 cm column of Sephadex G-50 was equilibrated with 20 mM NaCl - 20 mM Tris-HCl buffer (pH 7.6) and loaded with 1 ml of normal rat serum. Elution was carried out by running the same solution at 5° and fractions were collected every 0.7 ml eluant. A 50-µl aliquot of each fraction was added to the incubation mixture of a final volume of 180 µl as described in Material and Method and radioactivity of ^{3}H-TTP incorporated into DNA in each tube after 15 min of incubation was measured.

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while the outside fluid of it retains 27 % of the original activity. A series of repeated experiments has indicated that the ratio of activity of high-molecular component to that of low-molecular component in the serum is nearly 4:6.

The low-molecular component (outside fluid of 1:1 dialysis) was shown to be thermostable, while the high-molecular component (inside fluid of 1:∞dialysis) completely lost its activity after heating at 100° for 5 min.

The stimulatory activity of the outside fluid of 1:1 dialysis (low-molecular component) was found to be resistant to pronase digestion (2 mg pronase E/ml, at 37° for 1 h). The activity of it was not lost by treating the fluid with activated charcoal. These results seem to suggest that the active principle of low-molecular nature may not be related to either peptides or nucleotides.

When the whole serum was subjected to gel-filtration through Sephadex G-50, about 50 % of the activity was found in a macromolecular fraction and the rest of it was recovered in a small-molecular fraction as evidenced in Fig. 3, supporting the above described dialysis results.

Since the serum factors can stimulate the ^{3}H-TTP incorporation into DNA in the isolated nuclear system without a lag period in contrast to the serum effect on the cultured cells (11), the stimulated RNA or protein synthesis may not be involved in the activation mechanisms.

Stimulation of DNA synthesis by the rat serum may not be limited to the liver nuclei because Shimada and Terayama (19) has recently confirmed that the DNA synthesis in the isolated nuclei prepared from both newborn and adult rat brains can likewise be stimulated by the rat serum. The stimulatory factor(s) seems to be present also in the bovine and calf sera. Isolation and identification of the serum factors as well as the mechanisms of action of them are now in progress in our laboratory.

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