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Effect of Ginseng Saponins on Nuclear Ribonucleic Acid (RNA) Metabolism. I. RNA Synthesis in Rats treated with Ginsenosides

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The effect of ginsenosides purified from Ginseng (the roots of *Panax ginseng* C.A. Meyer) on nuclear ribonucleic acid (RNA) synthesis was investigated. Four hours after intraperitoneal injection of each of the ginsenosides (-Rb_1, -Rc, and -Rg_1) at a dose of 5 mg per 100 g body weight, incorporation of ^{3}H-orotic acid into nuclear RNA of rat liver was determined. The ginsenosides showed different activities: Rb_1 increased and Rc decreased the incorporation, and Rg_1 did not affect it. Furthermore, the effect of ginsenoside-Rb_1 and -Rc on RNA polymerase activity was investigated 3 hr after the intraperitoneal administration. Rb_1 enhanced and Rc repressed the enzyme activity, the results being consistent with those on the incorporation study. Both purified ginsenosides did not show any effect on RNA polymerase activity when added *in vitro*.

An extract of the roots of *Panax ginseng* C.A. Meyer has been reported to stimulate various metabolic reactions and it was also known that most of the active preparations contained saponins as main constituents.[2-4] In our laboratory a series of study has been undertaken on the effect of purified saponins in Ginseng to investigate whether such stimulating activity is definitely due to the saponins, or to other minor components. Sakakibara, *et al.* reported that most of the saponins purified from Ginseng enhanced markedly the incorporation of ^{14}C-acetate into liver and serum cholesterol.[5]

The present paper deals with the effect of three purified saponins (ginsenoside-Rb_1, -Rc and -Rg_1) of Ginseng on the synthesis of nuclear ribonucleic acid (RNA) in liver. Oura, *et al.* observed that a partially purified extract of Ginseng increased the incorporation of labeled precursors into liver nuclear RNA 4 hr after a single dose of intraperitoneal injection into rats.[6,7] The increased specific radioactivity of the nuclear RNA was not due to the change in the pool size of endogenous and soluble nucleotides,[6] and the ratio of RNA to DNA in the nuclei isolated at various intervals after the treatment was constant at the level of the nuclei from normal rats.[7] It was further shown that the extracts increased the activity of RNA polymerase [EC 2.7.7.6] assayed *in vitro*.[8]

The authors will report different effect of three purified ginsenosides on the incorporation of ^{3}H-orotic acid into nuclear RNA and on the activity of RNA polymerase.

Materials and Methods

Animals——Male rats weighing 150—200 g were used. The rats were fasted for about 18 hr prior to experiments.

1) Location: *1-5-8, Hatanodai, Shinagawa-ku, Tokyo.*
2) H. Oura, *J. Japan. Biochem. Soc.*, **45**, 804 (1973).
3) S. Hiai and H. Oura, *Protein, Nucleic acid and Enzyme*, **18**, 333 (1973).
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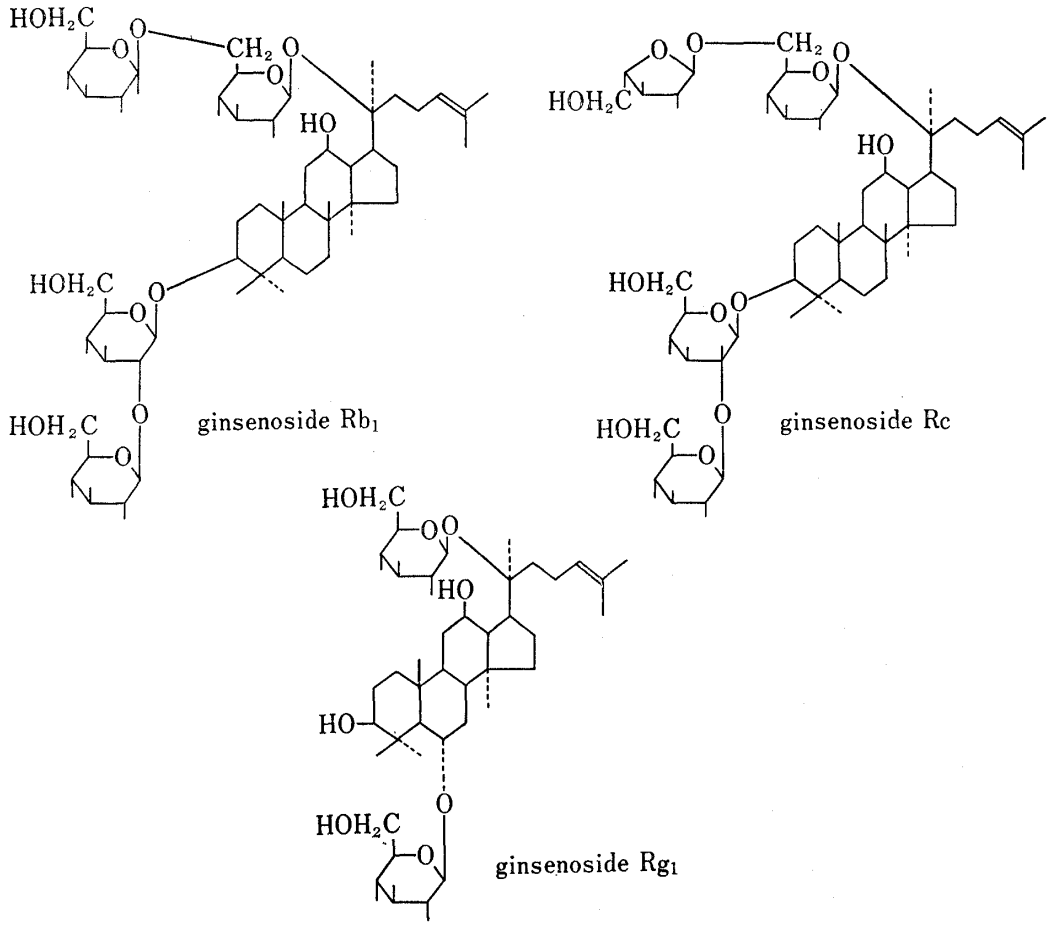

Chart 1. Structures of Ginsenosides used in This Study

Saponins——Ginsenoside-Rb_1, -Rc, and -Rg_1 were isolated and purified from Ginseng. All the preparation employed in this study was found to be pure by chemical and physicochemical analyses (Chart 1).[9-11]

Radiochemicals——5-^{3}H-orotic acid (17.9 Ci/mmole) was obtained from Radiochemical Center, England. 5-^{3}H-CTP (26.2Ci/mmole) was a product of New England Nuclear Corp., U.S.A.

General Procedures for Assay of Radioactivity of Liver Nuclear RNA in Saponin-treated Rats——Each ginsenoside (5 mg/100 g body weight) was given to rats intraperitoneally in saline solution. Control rats were treated with the equal volume of saline. The rats were killed 4 hr after administration of the ginsenoside. ^{3}H-ortic acid (10 μCi/100 g body weight) was injected intraperitoneally into animals 20 min prior to sacrifice. Livers were removed and the nuclei were separated as described below. Purified nuclei were washed with 0.6 N perchloric acid, ethanol and ether. To the residues was added 0.5 N KOH and the mixture was incubated at 37° for 18 hr, followed by acidification to remove deoxyribonucleic acid (DNA) and proteins as precipitates. After centrifugation the supernatant was neutralized with KOH. Radioactivity incorporated into nuclear RNA and the amount of RNA were determined using aliquots of this supernatant.

Separation of Nuclei of Rat Liver——The outline of the procedure is shown in Chart 2. Livers were washed with cold 0.25 M sucrose containing 3.3 mM $CaCl_2$ and minced with scissors. The mince was then homogenized with 3 volumes of the same solution in a Potter's homogenizer with a glass pestle and centrifuged at $1000 \times g$ for 10 min. The sediment was homogenized with 3 volumes of 0.25 M sucrose–3.3 mM $CaCl_2$ in a Potter's homogenizer with a Teflon pestle. The homogenates were filtered through 4 layers of gauze. Eight volumes of 2.2 M sucrose was added and the mixture was centrifuged at $40000 \times g$ for 1 hr to sediment the nuclei. The purified nuclei preparation had a ratio of RNA to DNA of 0.20.

Preparation of Lyzed Nuclear Enzyme——Preparation of RNA polymerase was carried out by the method reported by Oura, *et al.*[4] The purified nuclei were suspended in 0.02 M Tris-HCl buffer, pH 7.4, containing sodium deoxycholate at a final concentration of 2.0% and kept at room temperature for 30 min with occa-

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liver
↓ washed with 0.25 M sucrose–3.3 mM $CaCl_2$ (solution A) and minced with scissors
liver mince
↓ homogenized with 3 volumes of solution A in a Potter's homogenizer with a glass pestle and centrifuged at $1000 \times g$ for 10 min
precipitates
↓ homogenized with 3 volumes of solution A in a Potter's homogenizer with a Teflon pestle
homogenates
↓ filtered through 4 layers of gauze
supernatant fluid
↓ 8 volumes of 2.2 M sucrose was added and centrifuged at $40000 \times g$ for 1 hr
sediment: nuclei preparation

Chart 2. Separation of Rat Liver Nuclei

sional shaking. After centrifugation at $6000 \times g$ for 5 min, the supernatant was used for assay of RNA polymerase.

Assay for Activity of RNA Polymerase——The activity of the enzyme was measured from radioactivity of labeled cytidine triphosphate (CTP) incorporated into RNA fraction. The reaction mixture (1.0 ml) contained 1 μmole of Tris–HCl buffer, pH 8.0, 8 μmoles of β-mercaptoethanol, 12 μmoles of $MgCl_2$, 20 μmoles of KCl, 250 μmoles of ammonium sulfate, 4 μmoles of adenosine, guanosine and uridine triphosphates, 2.5 μCi of ^{3}H-cytidine triphosphate (26.2 μCi/μmole) and an enzyme preparation. The reaction mixture was incubated at 37° for 15 min. The reaction was stopped by adding 2.5 ml of chilled 10% TCA. Acid insoluble precipitates were washed with 5% TCA, ethanol and ethanol–ether. RNA was extracted twice with 10% NaCl at pH 8.0 in a boiling water bath under the addition of carrier RNA. To the extracts 2 volumes of ethanol were added. Precipitated RNA was washed with 65% ethanol, 99% ethanol and ether, successively. The dried preparation was dissolved in 0.4 ml of distilled water and the radioactivity was measured.

Radioactivity Measurements——Radioactivity was counted in a Beckman scintillation spectrometer with a slightly modified Bray's solution, the composition of which was as follows: one liter of the solution contained 50 ml of methanol, 10 ml of ethyleneglycol, 60 g of naphthalene, 4 g of 2,5-diphenyloxazole, 0.2 g of 1,4-bis-[2-(5-phenyloxazolyl)]-benzene and dioxane.

Protein Determination——Lowry's method[12] was employed.

Results

Effect of Ginsenoside-Rb_1, -Rc and -Rg_1 on the Incorporation of ^{3}H-orotic Acid into Nuclear RNA

The *in vivo* incorporation of ^{3}H-orotic acid into nuclear RNA was studied using rats treated with various ginsenosides of different structure, and the results are shown in Table I.

Table I. Effect of Ginsenoside-Rb_1, -Rc and -Rg_1 on Incorporation of ^{3}H-orotic acid into Liver Nuclear RNA

Rats	^{3}H-orotic acid incorporated	
	cpm/unit of OD_{260} (mean ± S.E.)	%
Control (8)	4666.9±366	100.0
Rb_1-treated (6)	5484.6±416	117.5
Rc-treated (6)	3999.7±459	85.7
Rg_1-treated (5)	4882.7±535	104.6

Figures in parentheses indicate the number of animals.

12) O.H. Lowry, M.J. Rosenbrough, A.L. Farr, and R.L. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

Ginsenoside-Rb_1 increased the incorporation of the labeled precursor over the control by 17.5%, whereas ginsenoside-Rc decreased the incorporation by 15%. Little effect could be detected with ginsenoside-Rg_1. It is interesting that different effect was observed with ginsenosides of different structure, and that the ginsenoside did not always stimulate the RNA synthesis in liver.

Effect of Ginsenoside-Rb_1 and -Rc on the Activity of RNA Polymerase *in Vivo*

Since it was reported by Oura, *et al.* that a partially purified extract of Ginseng increased the activity of RNA polymerase when injected into rats,[8] the effect of purified ginsenoside-Rb_1 and -Rc on the activity of this enzyme was investigated. The preparation of RNA polymerase was obtained as described by Oura, *et al.*,[8] and the enzyme assay was devised to eliminate a self-adsorption and to assure the reproducibility, being described in "Materials and Methods." Characterization of the enzyme preparation are shown in Table II and Fig. 1.

Table II. Conditions for RNA Polymerase Reaction

Conditions	^{3}H-CMP incorporated	
	cpm/mg protein	%
Complete	29520	100
Minus ATP	9492	32
Minus UTP	10544	36
Minus GTP	9592	33
Minus ATP, UTP, GTP	2392	8
Plus DNase[a]	72	0.25

a) preincubated with 400 μg of DNase at 37° for 10 min

It is apparent that the enzyme activity depends on the endogenous DNA in the preparation, and on the four ribonucleoside triphosphates as reported by Oura, *et al.*[8] (Table II). Fig. 1 demonstrates that the RNA polymerase activity was directly dependent on the amount of protein in the enzyme preparation up to 2.5 mg.

Rats were injected with ginsenoside-Rb_1 or -Rc at the dose of 5 mg per 100 g body weight, and after 3 hr liver nuclei were isolated and treated with deoxycholate as described in "Materials and Methods." RNA polymerase activity was assayed with the lyzed nuclear enzyme preparations of liver. As presented in Table III, Rb_1 increased the RNA polymerase activity over the control by 33% and Rc decreased it by 26%, the evidence being parallel to the one shown in Table I. Therefore, it was suggested that the enhancement or the depression of RNA synthesis observed by administration of the ginsenosides was principally accounted for by the change in RNA polymerase activity.

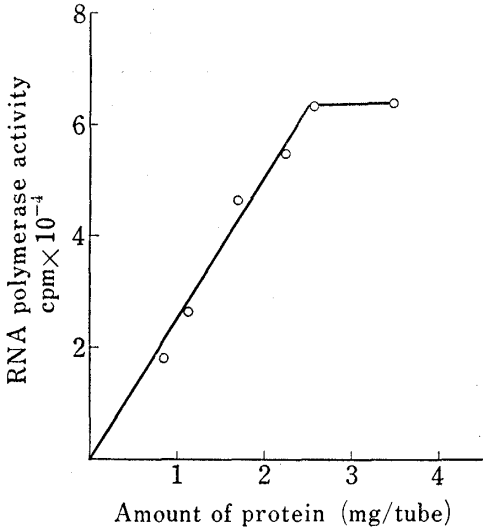

Fig. 1. Relationship between the Amount of Protein in Lysed Nuclear Enzyme Preparation and RNA Polymerase Activity

Lysed nuclear enzyme was prepared from livers of normal rats and enzyme activity was assayed as described in "Materials and Methods". RNA polymerase activity is represented as ^{3}H-CMP incorporated into RNA.

Effect of Ginsenoside-Rb_1 and -Rc on the Activity of RNA Polymerase *in Vitro*

In order to study a direct effect of the ginsenosides on RNA polymerase activity, Rb_1 or Rc was added to the reaction mixture for the assay of this enzyme activity. The results

are shown in Table IV. Neither ginsenoside-Rb_1 nor -Rc gave an effect on the activity measurement.

Table III. *In Vivo* Effect of Ginsenoside-Rb_1 and -Rc on RNA Polymerase Activity

Rats	^{3}H-CMP incorporated into RNA		
	cpm/mg of protein (mean	S.E.)	%
Control (6)	26680	1535	100.0
Rb_1-treated (4)	35390	2519	133.2
Rc-treated (4)	20830	2564	74.3

Figures in parentheses indicate the number of animals.

Table IV. *In Vitro* Effect of Ginsenoside-Rb_1 and -Rc on RNA Polymerase Activity

Addition		^{3}H-CMP incorporated	
		cpm/mg protein	%
None		24503	100
Rb_1	1 μg	23860	97
	10 μg	23405	96
	100 μg	23848	97
	500 μg	25159	103
Rc	1 μg	26329	107
	10 μg	25369	104
	100 μg	26963	110
	500 μg	22749	93

Nuclear enzyme preparation was preincubated with each ginsenoside at 37° for 15 min.

Discussion

The effect of purified ginsenoside-Rb_1, -Rc and -Rg_1 on the synthesis of nuclear RNA was investigated, and it was shown that Rb_1 stimulated the incorporation of ^{3}H-orotic acid into RNA when administered to rats. Therefore, it would be concluded that the enhancement of RNA synthesis upon injection of a partially purified extract of Ginseng reported by Oura, *et al.*[6,7] was mainly due to ginsenoside-Rb_1, which was a predominant constituent in their preparations. It is of interest that ginsenoside-Rc produced a decreased synthesis of nuclear RNA, and that ginsenoside-Rg_1 showed no effect on RNA synthesis. Such variabilities among the ginsenosides purified from Ginseng in their effect on cellular metabolism have been also observed on cholesterol metabolism[5] and on protein synthesis.[13] Accordingly, many biochemical effect obtained by a crude extract of Ginseng containing undoubtedly various kinds of saponins should be reinvestigated using each of the purified ginsenosides. It has not yet been possible to clarify a relationship between the activity of ginsenosides and their structures, and the discussion on this point should await further studies dealing with the effect of different ginsenosides on various metabolic reactions.

The present study has demonstrated that the effect of ginsenoside-Rb_1 and -Rc on the synthesis of nuclear RNA occurrs as the result of the changes in RNA polymerase activity. However, it has not been elucidated whether those changes are based on the increase or decrease

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in the actual amount of RNA polymerase, or they are due to the activation or inhibition of the enzyme. Immunochemical assay for this enzyme protein will be necessary to discriminate between the two possibilities. From their findings that actinomycin D and puromycin could not abolish a stimulating effect on RNA polymerase by the extracts of Ginseng, Oura, *et al.* suggested an enzyme activation rather than an induction in its synthesis.[8]

Since the purified ginsenosides did not affect RNA polymerase activity *in vitro*, their *in vivo* effect on this enzyme reported here would be produced through some intermediate agents which might include some of the hormones. Estradiol,[14] growth hormone[15] and testosterone[16] have been reported to increase the activity of RNA polymerase in uterus, liver and muscle, respectively.

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