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Biochemical Studies on the Chemical Components of Korean Ginseng (II)

Effects of Ginseng Components on the Activity of RNA Polymerase

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Abstract

Ginseng extracts were fractionated into several fractions with various organic solvents, and the effects of these fractions on the activity of RNA polymerase were examined. Fractions which showed positive effect on the activity of RNA polymerase were obtained both from white ginseng and red ginseng. For white ginseng the components which have shown a positive effect on RNA polymerase were found in total methanol extracts, the residual aqueous solution from ethyl acetate extraction and the methanol insoluble fraction of the above solution, whereas for red ginseng the positive components were found in total methanol extracts and in ethyl ether extracts. These findings suggest that the ginseng components which have positive effect on RNA polymerase be composed of polar and nonpolar moieties, which may be cleaved into two parts during the processing of red ginseng.

Many workers observed that extract of ginseng exerted a stimulatory effect on the synthesis of RNA in animal tissues[1]. Oura's works are very interesting on this point of view. He has observed that intraperitonial administration of ginseng extract not only induced increased activity of RNA polymerase, but also concurrently raised the level of cyclic AMP *in vivo*[2]. Oura's work suggests that some components behave like a hormone. Specifically he concluded that an active ginseng component seems to have an ACTH-inducing action.

We have partially purified the RNA polymerase from bovine liver and attempted to study if any component of ginseng extract increases the activity of RNA polymerase *in vitro*. Our purpose in this work was to obtain an evidence whether a component of ginseng extract increases the activity of RNA polymerase directly or indirectly. If there is a component which acts directly on RNA polymerase we will be able to design an experiment for scrutinizing the action mechanism of the active component on RNA polymerase.

Materials and Methods

1. Materials

Six-year old white ginseng was provided by the Central Research Institute, Office of Monopoly, Korea and red ginseng was purchased from Office of Monopoly.

2. Methods

Preparation of RNA Polymerase. Enzyme was prepared from bovine liver by the method of Weiss[3]. The fresh tissue (150 g) was homogenized in 0.25 M sucrose solution containing 0.001 M magnesium chloride. The homogenate was filtered through two layers of gauze, and the filtrate was centrifuged for 6 minutes at 600 ×g at 4°. The supernatant was discarded. The pellet was resuspended in 20 volumes of homogenizing solution and rehomogenized. This preparation was washed with isotonic sucrose solution(0.25 M) and kept for 10 minnutes at 0°. Lysed nuclei were centrifuged at 10,000 ×g and the supernatant was discarded. Resulting pellet was suspended in 64 m*l* of 0.05 M Tris buffer and 16 m*l* of 2 M KCl was added dropwise. After removing the water, aggregate obtained was suspended in 32 m*l* of 0.05 M Tris buffer. Protein content of the final solution was 0.12 mg/m*l*.

Fractionation of Ginseng Extracts. The same fractions which were prepared for our works on aminoacyl-tRNA synthetase were used(see elsewhere in this issue).

Enzyme Assay. Enzyme assay was carried out according to Weiss[3]. 8-^{3}H-ATP was purchased from the Radiochemical Center, England. Enzyme reaction mixture contained 100 μmoles of Tris buffer (pH 8.1), 5 μmoles of magnesium chloride, 10 μmoles of mercaptoethanol, 2 μmoles of each GTP, UTP, CTP, 0.1 μmoles of ^{3}H-ATP(5×10^6 cpm/μmoles), 1 m*l* of enzyme solution, and 20 μl of ginseng extract. Control was the complete enzyme assay mixture except that the ginseng extract was omitted. The reaction mixture was incubated for 20 minutes at 37°. The reaction was stopped by adding 5% trichloroacetic acid. The labeled RNA was precipitated with carrier RNA. The precipitated RNA was dissolved in 0.01 N ammonium hydroxide solution and its radioactivity was counted with liquid scintillation counter.

Results and Discussion

Effect of ginseng extract on the activity of RNA polymerase is given in Table I. Data represent the percent increase of activity compared to that of control. Fractions which exert positive effect to RNA polymerase were obtained both from white ginseng

Table I. The effect of several fractions of ginseng extract on the activity of RNA polymerase. Data are represented in percent increase of activity compared to that of control.

White Ginseng	Fraction	W_{H_2O}	W_1	W_3	W_4	W_5	W_{5-a}	W_{5-b}
	Percent increase of enzyme activity	0	+2374	−11.3	−3.2	+774	+19.4	−32.4
Red Ginseng	Fraction	R_{H_2O}	R_1	R_2	R_3	R_4		
	Percent incresse of enzyme activity	−45.2	+822	+851.6	−32.3	−3.2		

and red ginseng. For white ginseng, total methanol extract (fraction W_1), mother liquor of ethyl acetate extraction (fraction W_5), and the aqueous extract of fraction W_5 (fraction W_{5-a}) had the stimulatory effect. For red ginseng, whole methanol extract (fraction R_1) and ether extract of fraction R_1 (fraction R_2) had considerably high stimulatory effect. It was noteworthy that fraction W_1 and fraction W_5 of white ginseng had specially high stimulatory effect. Such observations suggest that active components of white ginseng should be rather polar substances. On the other hand, we can easily deduce from the fractionation steps of ginseng extract that the active components in red ginseng should have somewhat nonpolar character. From these results, we are able to presume that the possible active components in white ginseng had undergone some chemical modification in the course of processing for the preparation of red ginseng.

Oura and Hiai[2] found that administration of ginseng extract into the body of rat raised the level of cyclic AMP and RNA temporally within 30 minutes and the level decreased to normal value after 1 to 2 hours. However, this observation alone does not give sufficient solution whether the active components of ginseng extract stimulate the activity of RNA polymerase directly or indirectly. Our results obtained from *in vitro* experiment give the definite conclusion that the active components of ginseng extract stimulate the activity of RNA polymerase directly. Therefore we think that it is worthwhile to search an active factor for RNA polymerse, and to study the efficacy of ginseng components on the molecular level.

Acknowledgment

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한국 인삼 성분들에 관한 생화학적 연구(Ⅱ)

인삼 성분들이 RNA 중합효소의 활동성에 미치는 영향

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초 록

몇 가지 유기용매를 사용하여 인삼 추출액을 분별 분리하여 몇 개의 분획들을 얻어서 이들이 RNA중합효소의 활동성에 미치는 영향을 조사하였다. 백삼과 홍삼에서 모두 RNA 중합효소의 활동성에 양성적인 효과를 가진 분획들을 얻었다. 백삼의 경우 RNA 중합효소에 양성적인 효과를 미치는 성분들은 전체 메탄올 추출액 분획, 초산에틸 추출모액 및 이 모액의 수용성 분획들에서 확인되었고 홍삼의 경우에는 전체 메탄올 추출액 분획과 에테르 분획들에서 확인되었다. 이 사실은 중합효소에 양성적인 효과를 나타내는 성분들이 무극성 및 극성 부분들로 이루어져 있으며, 그들이 홍삼의 가공과정에서 두 부분으로 분해되었을 것이라는 것을 암시해 준다.