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

Effects of Components of Ginseng on the Activity of Aminoacyl-tRNA Synthetase

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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 aminoacyl-tRNA synthetase was examined. Fractions which showed positive effect on the activity of the aminoacyl-tRNA synthetase were obtained both from white ginseng and red ginseng. The total methanol extract of white ginseng and the ether extract from the total methanol extract of red ginseng gave positive results. Therefore, it may be presumed that the positive components have rather nonpolar nature.

Korean ginseng has long been regarded as an elixir of life. A number of papers were published over past fifty years on the chemical, physiological and pharmacological studies on the chemical components of ginseng. Most of the papers published in early period were concerned with the examination of physiological or morphological changes which appeared after the administration of ginseng extracts or ginseng powder into the animal body[1]. Recently, investigations on the properties of some chemical components of ginseng have been intensively carried out to elucidate the chemical structures of some components, such as saponins[2,3,4,5]. New biochemical or molecular biological approaches to study the specific effects of ginseng extracts of some saponins on particular metabolic pathways were also adopted by many workers [5~11]. Even with these approaches, many experiments were carried out still by administrating the ginseng extracts or particular components into the whole animal body and by analyzing the experimental animal from the point of view of some metabolic changes.

We have carried out this work to find out the chemical components of ginseng which stimulate the activity of aminoacyl-tRNA synthetase *in vitro*. By doing this

way, it should be possible to explain more definitely the efficacy of a ginseng component on the molecular level, and to elucidate its chemical properties.

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, Korea.

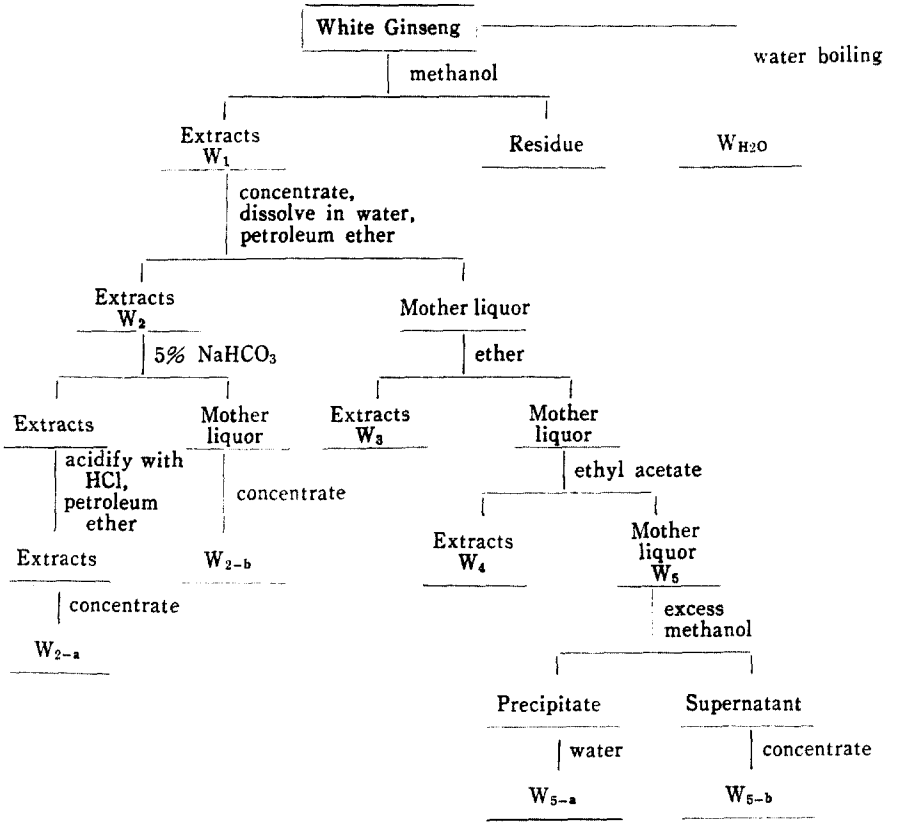
2. Methods

Preparation of Aminoacyl-tRNA Synthetase. This enzyme was extracted from bovine liver by the method of Hatfield *et al.*[12]. After 150 g of fresh tissue was minced very well, it was mixed with 3.5 volumes of the solution containing 0.05 M Tris-HCl buffer(*p*H 7.4), 0.35 M sucrose, 0.05 M magnesium chloride, 0.0025 M potassium chloride, 0.035 M potassium bicarbonate, and 0.5 mM mercaptoethanol, and the mixture was homogenized. Heavy residue was removed by centrifugation at 20,000×g, and the supernatant was centrifuged for 45 minutes at 105,000×g. Resulting supernatant was adjusted to 0.1 M with respect to Tris-HCl buffer and sodium chloride. This solution was mixed with the equal volume of DEAE-cellulose, and after thorough stirring of the mixture, DEAE-cellulose was removed. By adjusting this solution to 75% saturation with ammonium sulfate, precipitate was obtained. This precipitate was dissolved in 2 m*l* of the mixed buffer soution and stored at −25°. Protein content of this solution was 0.012 mg/m*l*.

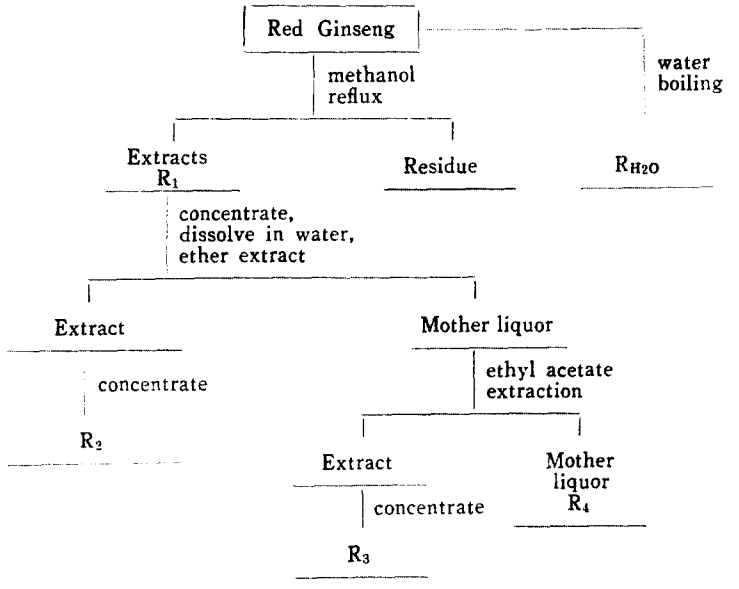
Preparation of ^{32}P-Pyrophosphate. ^{32}P-pyrophosphate was prepared by the method of Berg[13] with 3 mCi of ^{32}P-orthophosphate (radiochemical purity, 99%). ^{32}P-orthophosphate was purchased from Korea Atomic Energy Research Institute. Specific radioactivity of ^{32}P-pyrophosphate prepared was 1.8×10^4 cpm/μmole.

Fractionation of Ginseng Extract. White ginseng was extracted with methanol for 3 to 4 days using large scale Soxhlet apparatus. This methanol extract was concentrated as far as possible in rotary vacuum evaporator, and the resulting thick, brown-colored residue was dissolved in a little amount of water, and extracted successively with petroleum ether, ethyl ether, and ethyl acetate as shown in Scheme I. Ethyl acetate in mother liquor was removed until no more smell of ethyl acetate was detected. Onthe other hand, water extract of white ginseng was also prepared separately by boiling gently for 5 to 6 hours in water.

For red ginseng, its methanol extract was made by boiling it with methanol for about 48 hours in a flask attached with reflux condenser. After concentration of the



Scheme I. Fractionation of White Ginseng.



Scheme II. Fractionation of Red Ginseng.

extract to small volume in rotary vacuum evaporator, the residue was dissolved in a little amount of water and extracted successively with ethyl ether, and ethyl acetate as shown in Scheme II. On the other hand, water extract of white ginseng was also prepared separately by boiling gently for 5 to 6 hours in water.

Except for water extracts, extraction solvents were removed under reduced pressure from all extracts. Resulting residues were dissolved in small amount of water of methanol and used for enzyme assay components.

Enzyme Assay. The activity of aminoacyl-tRNA synthetase was measured by determining the extent of exchange reaction between ^{32}PPi and ATP by the method of Stulberg and Novelli[14]. Enzyme assay mixture contained 10 μl of enzyme solution, 100 μmoles of Tris-HCl buffer(pH 7.5), 5 μmoles of ATP, 5 μmoles of ^{32}PPi, 5 μmoles of magnesium chloride, 10 μmoles of serine, 50 μmoles of potassium fluoride, and 20 μl of each fraction of extract of ginseng in final volume of 1ml. Control was complete reaction mixture except that the ginseng extract was omitted. Reaction mixture was incubated for 15 minutes at 37°, and the reaction was stopped by adding 5% of trichloroacetic acid solution. The amount of ^{32}PPi incorporated into ATP was measured by determining the radioactivity adsorbed on Norit A. Radioactivity was measured with liquid scintillation counter

Results and Discussion

Effect of ginseng extract on the activity of aminoacyl-tRNA synthetase is shown in Table I. Data represent percent increase of activity compared to that of control. Although most of the fractions has shown inhibitory effect on the enzyme activity, methanol fraction of white ginseng(fraction W_1) has shown stimulatory effect of 21.2 % and ethyl ether fraction of red ginseng(fraction R_2) has shown slight stimulatory effect of no more than 1.8%. The fact that only methanol fraction of red ginseng has positive effect while the other fractions have negative effect suggests that active

Table I. The effect of several fractions of ginseng extract on the activity of aminoacyl-tRNA synthetase. Data are represented in percent incresase of the activity of enzyme compared to that of control.

White Ginseng	Fraction	W_{H2O}	W_1	W_{2-a}	W_3	W_4	W_5	W_{5-b}
	Percent increase of enzyme activity	−14.5	+21.2	−10.4	−0.8	−4.7	−8.1	−13.4
Red Ginseng	Fraction	R_{H2O}	R_1	R_2	R_3	R_4		
	Percent increase of enzyme activity	−16.9	−8.1	+1.8	−20.6	−10.2		

component may be more or less nonpolar substance. This idea is further supported by the fact that ether extract(fraction R_2) of methanol extract of red ginseng has positive effect. In addition, this also suggests that fraction R_2 contains a more nonpolar active component which is different from a component in fraction W_1. The difference between the active components of fractions W_1 and R_2 also suggest that the active component of fraction W_1 was probably undergone a chemical modification in the course of processing for the preparation of red ginseng.

When the active component which exerts positive effect on the activity of aminoacyl-tRNA synthetase was isolated from ginseng extract, it would be a very attractive work to study the detailed interaction between this component and the enzyme. The future work on this line will enable us to interpret the efficacy of ginseng on the biochemical and molecular biological basis.

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한국 인삼 성분들에 관한 생화학적 연구(I)

인삼 성분들이 아미노아실-tRNA 합성 효소의 활동성에 미치는 영향

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초 록

인삼의 메탄올 추출물을 몇 가지 유기용매를 사용하여 여러 분획으로 나누어서 이 분획들이 아미노아실-tRNA 합성효소의 활동성에 미치는 영향을 조사하였다. 백삼과 홍삼에 모두 아미노 아실-합성효소의 활동성에 양성적인 효과를 나타내는 분획을 얻었다. 백삼의 경우에는 전체 메탄올 추출물에 그 성분이 있으며, 홍삼의 경우에는 메탄올 추출물을 에테르로 추출한 분획에 그 성분이 있었다. 그러므로 이 효소에 양성적인 효과를 미치는 성분은 다소 무극성인 물질일 것으로 추측된다.