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Effect of Triol and Diol Fractions of Ginseng Saponin on Glutamine Transport into Rat Renal Cortical Mitochondria

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인삼의 Triol 및 Diol계 사포닌이 쥐의 신피질 미토콘드리아의 Glutamine 이동에 미치는 영향

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Abstract

Attempts were made if diol and triol fractions of ginseng saponin affect on glutamine transport into rat renal cortical mitochondria, swelling, phosphate dependent glutaminase activity, and consumption of oxygen. The following results were obtained. When mitochondrial preparation from rat renal cortex was incubated in medium containing ^{14}C-glutamine and either triol or diol fractions, radioactivity was shown to increase at both 10^{-6}% and 10^{-5}% triol fractions of ginseng saponin, but reduce in case of diol fraction. The remarkable acceleration of the rate of swelling of renal cortical mitochondria was observed in the presence of 10^{-1}% triol and diol fractions but no accerelation at lower concentrations. The activity of phosphate dependent glutaminase from renal cortical mitochondria was slightly activated at 10^{-2}% of triol fraction. However, there was no effect in case of diol fraction. Oxygen consumption by mitochondria from renal cortex was remarkably increased at concentrations of 10^{-5}% and 10^{-6}% triol fractions, but reduced in the case of diol fractions. On the basis of these observations it was concluded that triol fraction of ginseng saponin might increase the transport of glutamine into mitochondria by accelerating the respiratory chain and supplying additional energy to mitochondria, and physiological role of triol fraction was entirely different from that of diol fraction of ginseng saponin.

Introduction

Recent studies have suggested that the transport of glutamine across the inner membrane of renal mitochondria may play an important role in the regulation of renal glutamine deamination and ammonia production.[1-6] Mitochondrial glutamine transport[3, 7, 8] is not active[11] but carrier-mediated transport, since it is inhibited by, mercurials[1, 3], and glutamine analogue 6-diazo-5-oxo-L-norleucine[4, 22], and shows saturation kinetics[5].

Since renal glutamine deamidation is thought to occur mainly within the inner membrane-matrix compartment of renal mitochondria[3, 11, 12], the mitochondrial glutamine carrier occupies a central position in the conversion of cytoplasmic glutamine to ammonia and glutamic acid. Cumulative evidence in support of the physiological role of the mitochondrial glutamine carrier is derived from studies in which it was shown that glutamine transport capacity was elevated in mitochondria isolated from kidney of acidotic rats[1, 3] and dogs[5]. In addition, a recent report has shown that the activity of the mitochondrial glutamine carrier may be regulated by physiological concentration of α-ketoglutarate in the cell[4] and that alteration in level of the keto acid similar to that observed during acute metabolic acidosis led to significant deinhibition of glutamine carrier[4].

Mersalyl, an inhibitor of phosphate transport across the inner mitochondrial membrane, strongly inhibited the activation of glutaminase in intact mitochondria only in the presence of inhibitors of electron transport or of an uncoupler[8]. The activity of the mitochondrial glutaminase was strongly inhibited by avenaciolide or Bromocresol purple in the prescence of inhibitors of respiration or an uncoupler but not in their absence[7]. Such results suggest that this was caused by inhibition of glutamate efflux. However, the addition of a detergent removed this inhibition. In addition, anion such as phosphate[10, 14] and Mg^{2+}, Mn^{2+}, Ca^{2+}, and NH_3[15, 16] were also known to be responsible for activity[7, 13].

The aims of the present study were: 1) to see if there might be the effect of triol and diol fractions of ginseng saponin on transport of glutamine into mitochondria in connection with previous report[17] in which ^{14}C-glucose transport was elevated in the prescence of ginseng saponin[17], and 2) to check whether both fractions of ginseng saponin could swell mitochondria as previous report[18], and 3) to observe if both fractions of ginseng saponin could elevate the activity of glutaminase obtained from mitochondria in relation to previous papers in which the enzymatic activity was remarkably increased by saponin fraction[19], and finally, to see both of fraction might effect on oxygen consumption in isolated mitochondria for explanation on physiologically different roles of diol and triol fractions as described[20].

Materials and Methods

Materials:

Triol and diol fractions were obtained from Korean Ginseng and Tobacco Institute.

Rats (Sprague-Dawley, 200-300 g) were fed normal diet and given sufficient water, L-Glutamine, L-glutamic acid, rotenone, succinate, EDTA, Tris, ATP, ADP, NAD^+, glutamate dehydrogenase, glycine, and Triton X-100 were products of Sigma Chemical Co. PPO and POPOP were pur-

chased from Amersham. L-[U-^{14}C] glutamine was purchased from New England Nuclear. Other chemicals were reagent grade.

Methods:

Preparation of rat renal cortical mitochondria

Rat renal cortical mitochondria was obtained as previously described[5] with the exception that decapitation was used in place of injecting a bolus of potassium chloride intravenously and exsanguinating. Briefly, rats were killed by decapitation. The kidney were removed and placed in an ice-cold solution of 0.14M NaCl and 0.01M KCl. The renal cortex was seperated by Stadie-Riggs microtome. Three gram portions of cortex were placed in 15ml of ice-cold 0.3M sucrose/5mM HEPES/1mM EDTA and homogenized by Dounce homogenizer. The homogenate was centrifuged for 5 min at 700×g. The supernatant was centrifuged for 10 min at 8000×g. The pellet was resuspended in 0.3M sucrose/5mM HEPES and centrifuged in the same manner. The pellet from this centrifugation was again resuspended and centrifuged. The final pellet was resuspended in a appropriate volume of 0.3M sucrose/5mM HEPES. All steps in isolation were carried out at 0.4°C.

Integrity of mitochondria

It was done as described elsewhere.[21] Briefly, respiratory control ratio (state III/state IV) was checked using Na-glutamate as substrate and respiratory control ratio was turn out to be 5.31 ± 0.152, which was used as mitochondria source.

Assay of glutamine transport

0.1ml of mitochondrial solution (1.3mg protein/ml) was incubated with 0.9ml incubation medium containing 250mM sucrose, 20mM HEPES, 1mM succinate, 1mM EDTA, 25mM $MgCl_2$, 2.5mM K_2HPO_4, 2μg/ml rotenone (to inhibit glutamate oxidation), 0.4uCi L-[U-^{14}C] glutamate in the presence of diol and triol fractions for 0.5 min. After incubation, 0.1ml of incubation medium were pipetted as rapidly as possible onto Millipore filter (0.45μm pore size and 25mm diam.) that had been prewashed with solution containing 250mM sucrose and 20mM HEPES, pH 7.4 and rapidly vacuum filtered. After washing, mitochondria on filter paper was dried, and placed in 10ml of liquid scintillation cocktail for one night at 4°C and radioactivity was measured by liquid scintillation counter (Packard Tricard 300).

Mitochondrial swelling

The stock solution of mitochondria was diluted with 0.3M sucrose buffered to pH 7.4 with 0.02M Tris before spectrophotometric measurement at 520nm were taken. Final concentration of mitochondrial solution was 0.33mg protein/ml. The changes in optical density of three ml of the solution were checked in the presence of various concentrations of diol and triol fractions of ginseng saponin after 30 min incubation at 37°C. This technique for measuring mitochondrial

swelling is a slight modification of that described elsewhere[25].

Enzyme assays

The phosphate-dependent glutaminase (E.C. 3.5.1.2) was prepared from isolated mitochondria as described previously[22] and the activity of the enzyme was determined as described elsewhere[23] except that various concentrations of diol and triol fraction of ginseng saponin. The activity was expressed in terms of n mole of glutamate produced per mg protein per min.

Assay of oxygen consumption

Oxygen consumption was measured with Clark oxygen electrode to check decrease in oxygen partial pressure as described elsewhere[23]. For these measurements medium contained 16.7mM Na-glutamate, 135mM sucrose, 58mM KCl, 1.3mM KH_2PO_4, 8.7mM K_2HPO_4, 5mM $MgSO_4$ and various concentration of diol and triol fraction of ginseng saponin and was added to 1mg protein/ml of the mitochondrial solution.

Protein determination

The protein content of mitochondrial preparation was determined by the biuret reaction described elsewhere[24]. A standard curve was made from bovine serum albumin. The protein determination was carried out to check the reproducibility of isolating the mitochondria.

Results and Discussion

Fig. 1 shows the formation and accumulation of labeled product produced from ^{14}C-L-glutamine by mitochondria from rat renal cortex. The system appears to follow saturation kinetics. Such saturation trend is in good agreement with previous paper[6] and suggests that there must be glutamine carrier in mitochondria. According to previous paper[5] however, glutamate is

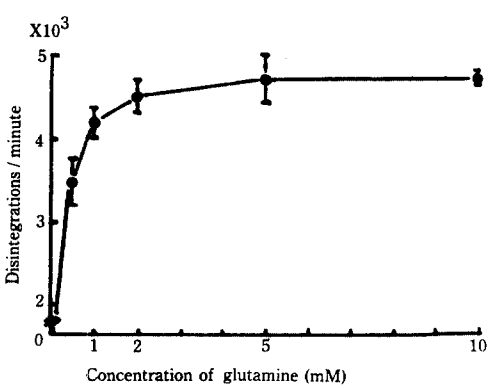

Fig. 1. Formation and accumulation of product formed from various concentrations of L-glutamine in medium.

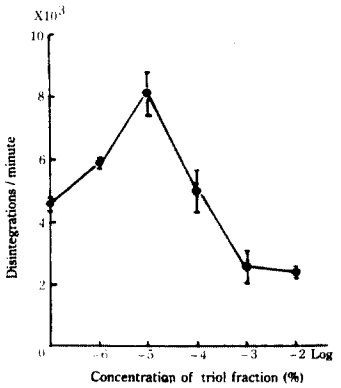

Fig. 2. The effect of triol fraction of ginseng saponin on transport of glutamine into inner mitochondrial compartment.

transported out of mitochondria into the medium, resulting in a steadily increasing concentration of glutamate in medium. Incubation of mitochondria with ^{14}C-glutamine for 0.5 min seems to minimize transport of glutamate from mitochondria into medium showing saturation curve considering previous claims[7], and there must be little amount of glutamate in medium which we did not check, as previous paper[6].

Fig. 2 shows the effect of different concentrations of ginseng saponin triol fraction on glutamine transport from medium into mitochondria. Remarkable increase in glutamine was observed at 10^{-6} and 10^{-5}% compared to that of the control but inhibitory effect at more than 10^{-3}%. In chronic acidosis there is a fourfold increase in the uptake of glutamine but not α-ketoglutarate, glutamate or acetate[1]. Even in 3 hr acidosis[1], there is increase in uptake of glutamine, too.

They suggest that carrier for glutamine does exist in the inner membrane and an adaptive

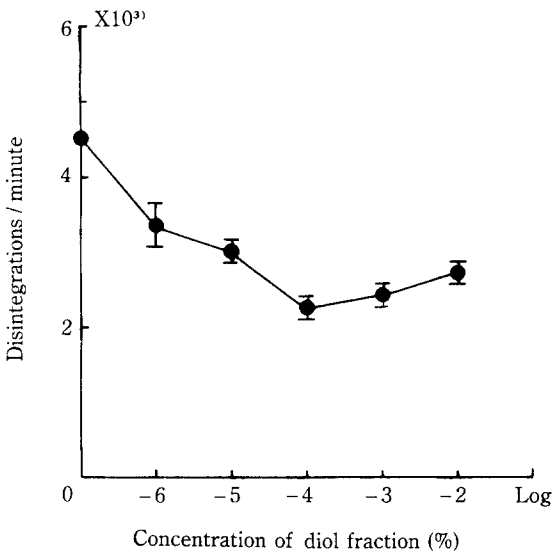

Fig. 3. The effect of diol fraction of ginseng saponin on transport of glutamine into inner mitochondrial compartment.

Table 1. The effect of triol fraction of ginseng saponin on absorbance at 520nm of mitochondrial suspensions. Mitochondria, representing 1mg protein, were suspended in 3ml of 0.3M sucrose. Samples were incubated at pH 7.4 for 30 min at 37°C. The values are means ±SE.

Concentration of triol fraction (%)	Absorbance 520		Relative absorbance** at t_{30}
	t_0*	t_{30}*	
0	0.500	0.478±0.006	96
10^{-5}		0.465±0.006	93
10^{-4}		0.468±0.003	94
10^{-3}		0.465±0.001	93
10^{-2}		0.433±0.000	87
10^{-1}		0.094±0.003	19

* t number: Incubation time (min).
**The relative absorbances are expressed assuming the absorbance of control at t_0 is 100.

increase in the capacity of this carrier is probably the primarily responsible for the increased ammonia production in metabolic acidosis.

There might be one possibility that the increase in transport of glutamine in the presence of triol fraction might be due to changes in membrane as described previously[17].

As shown in Fig. 3, concentrations ranging from 10^{-6} to 10^{-2}% of diol fraction is shown to inhibit transport compared to that of the control. Clearly, the role of the triol fraction is entirely different from that of diol fraction as far as glutamine transport is concerned and trends are in agreement with previous paper[20].

Table 1 and 2 shows the effects of triol and diol fractions of ginseng saponin on swelling of renal mitochondria. Above 10^{-2}% there was a remarkable swelling whereas below 10^{-3}% there was no observable change in swelling. However, the mechanism for swelling is not clear con-

Table 2. The effect of diol fraction of ginseng saponin on absorbance at 520nm of mitochondrial suspensions. Mitochondria, representing 1mg protein, were suspended in 3ml of 0.3M sucrose. Samples were incubated at pH 7.4 for 30 min at 37°C. The values are means ±SE.

Concentration of diol fraction (%)	Absorbance 520		Relative absorbance** at t_{30}
	t_0*	t_{30}*	
0	0.500	0.409±0.004	82
10^{-5}		0.388±0.008	78
10^{-4}		0.423±0.010	85
10^{-3}		0.395±0.013	79
10^{-2}		0.277±0.014	55
10^{-1}		0.081±0.004	16

*t number: Incubation time (min).
**The relative absorbances are expressed assuming the absorbance of control at t_0 is 100.

Table 3. The effect of triol fraction of ginseng saponin on phosphate dependent glutaminase in renal cortical mitochondria *in vitro*. The reaction mixture contained (total volume, 1.0ml) 0.15M KH_2PO_4, 0.2mM EDTA, 50mM Tris (pH 8.6), 20mM glutamine, various amounts of triol fraction and mitochondrial preparation (0.2mg protein). The values are means ±SE.

Concentration of triol fraction in the solution (%)	Phosphate-dependent glutaminase activity (n moles/mg protein/min)*	Relative activity**
0	155.7±1.50	100
10^{-6}	154.4±0.58	99
10^{-5}	154.7±1.17	99
10^{-4}	155.0±3.77	100
10^{-3}	168.7±0.09	108
10^{-2}	188.0±0.67	121

*The activity of enzyme was expressed as the amount of glutamate formed from glutamine per min per mg protein under the above conditions.
**The relative activities are expressed assuming the activity of control is 100.

Table 4. The effect of diol fraction of ginseng saponin on phosphate dependent glutaminase in renal cortical mitochondria *in vitro*. The reaction mixture contained (total volume, 1.0ml) 0.15M KH_2PO_4, 0.2mM EDTA, 50mM Tris (pH 8.6), 20mM glutamine, various amounts of diol fraction and mitochondrial preparation (0.2mg protein). The values are means ±SE.

Concentration of diol fraction in the solution (%)	Phosphate-dependent glutaminase activity (n moles/mg protein/min)*	Relative activity**
0	182.7±2.85	100
10^{-6}	166.2±0.20	91
10^{-5}	168.2±1.55	92
10^{-4}	174.3±1.40	95
10^{-3}	162.3±1.75	89
10^{-2}	198.6±0.75	109

*The activity of enzyme was expressed as the amount of glutamate formed from glutamine per min per mg protein under the above conditions.
**The relative activities are expressed assuming the activity of control is 100.

sidering that the rate of swelling of mitochondria from acidotic rats is more rapid than that of mitochondria from normal rats in case of incubation in an isotonic medium containing phosphate[25].

Glutaminase (L-glutamine amidohydrolase, E.C. 3.5.1.2) is an intramitochondrial enzyme and consists of two isoenzymes, one phosphate dependent and the other phosphate independent. Phosphate dependent glutaminase is probably more important than phosphate independent glutaminase since its level in kidney is about ten times greater than phosphate independent glutaminase[26] and has been most studied in kidney where its principal function is to generate ammonia from glutamine. Accordingly, the effect of triol and diol fractions of ginseng saponin on phosphate dependent glutaminase activity was checked *in vitro* and there was a little effect only in case of 10^{-2}% of triol fraction. However, there was no effect in the presence of diol fraction (Tables 3 and 4). In the absence of phosphate, phosphate dependent glutaminase exist as a catalytically inactive protomer[14]. The addition of phosphate results in both dimerization and activation of the glutaminase suggesting that *in vivo* regulation may be achieved by molecule manipulation of a monomer-dimer equilibrium. Although the nonspecificity of glutaminase's requirement for activator is atypical for enzymes regulation by different tissues, which may be required by the tissue specific functions of glutaminase. In this connection, saponin fraction might be substituted for ligand as described above.

Oxygen consumption by mitochondria was measured in the presence of triol and diol fractions of ginseng saponin, respectively (Figs. 4 and 5). 10^{-6}% and 10^{-5}% of triol fractions seeming to be physiologically meaningful were shown to increase oxygen consumption, respectively whereas there was inhibitory effect at higher concentrations. But diol fraction tended to inhibit oxygen consumption.

Our cumulative results suggest that the acceleration in the rate of transport of glutamine from medium to mitochondria by triol fraction could be due to higher consumption of oxygen by triol fraction, which is between 10^{-6}% and 10^{-5}%, excluding that acceleration in the rate of swelling of isolated mitochondria play a role in the greater activation of glutaminase by triol fraction as

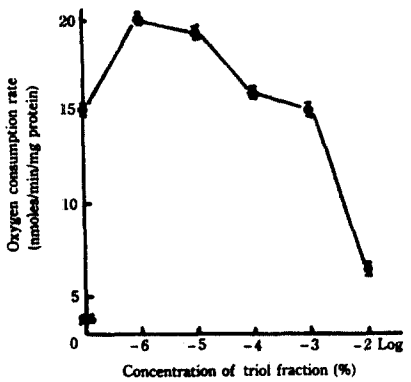

Fig. 4. The effect of diol fraction of ginseng saponin on rate of oxygen consumption by renal cortical

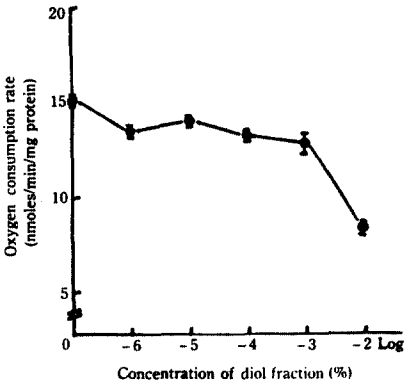

Fig. 5. The effect of triol fraction of ginseng saponin on rate of oxygen consumption by renal cortical mitochondria.

suggested in case of phosphate[25]. Finally, role of triol and diol fractions have different character clearly, suggesting that ratio of them seems to be critically important in mixture as far as mixture of them is used in experiments.

要 約

쥐의 신피질에서 분리한 미토콘드리아의 glutamine 이동에 미치는 인삼사포닌(triol 계와 diol 계)의 영향을 조사하고 인삼 사포닌에 의한 미토콘드리아 막의 swelling 여부와 phosphate dependent glutaminase 의 활성 및 미토콘드리아의 산소 소모율에 미치는 영향을 관찰하여 다음과 같은 실험결과을 얻었다.

1. 신피질 미토콘드리아를 ^{14}C-glutamine 과 여러가지 농도의 triol 계 인삼사포닌 수용액 또는 diol 계 인삼사포닌 수용액에 방치한 결과 glutamine 이 미토콘토리아 내부로 이동되어 축적된 산물의 양은 triol 계의 경우, 그 농도가 10^{-6}%및 10^{-5}%일때 대조군보다 증가하였고 diol 계의 경우는 억제되었다.

2. 신피질 미토콘드리아를 인삼사포닌(triol 계 또는 diol 계)이 포함된 등장완충용액에 방치하였을 때 사포닌의 농도가 10^{-1}% 일때는 triol 계 및 diol 계 모두 미토콘드리아를 현저하게 swelling 시켰으나 이보다 낮은 농도에서 대조군과 거의 비슷한 양상을 나타내었다.

3. 신피질 미토콘드리아 phosphate dependent glutaminase 의 활성은 triol 계의 경우 그 농도가 10^{-2}% 일때 효소활성이 약간 증가하였으나 diol 계의 경우 거의 변화가 없었다.

4. 여러가지 농도의 triol 계 또는 diol 계의 인삼사포닌 수용액에 신피질 미토콘드리아를 방치한 후 산소 소모율을 측정한결과 triol 계의 경우 그 농도가 10^{-6}%－10^{-5}% 일때 대조군보다 현저하게 증가하였으나 diol 계의 경우는 오히려 감소 하였다.

이상과 같은 실험결과는 triol 계 인삼사포닌이 신피질 미토콘드리아의 phosphate

dependent glutaminase에는 큰 영향을 미치지 못하나 호흡계를 촉진하여 에너지 공급을 원활하게 하므로써 glutamine의 이동을 증가시키는 것으로 생각된다.

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