IDENTIFICATION AND SOMNOGENIC EFFECTS OF SOMEN - γ - GLUTAMYL OLIGOPEPTIDES FROM *PANAX GINSENG*

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INTRODUCTION

Our studies were focused on nonprotein amino acids and peptides in the water extract of Panax species[1]. The physiological activities of some oligopeptides were evaluated.

The materials used for the present investigation were collected from different places. *Panax ginseng* (C.A. Meyer) was collected from Ji'an ginseng farm of Ji'an county, Jilin province. Two samples were used, the 5th grade and ungraded, for our investigation. The sample were the main roots with some fibrous roots and rhizome of the plant. *Panax notoginseng* (F.H. Chen) was collected from Yunnan and Guangxi province of Southwestern China respectively.

From the free amino acides of *Panax ginseng* (Jilin, China), an inhibitory neurotransmitter, γ - aminobutyric acid(GABA)[2], a neuroexcitotoxic and hemostatic nonprotein amino acid, β - oxalo - L - α, β - diaminopropionic acid (β - N - ODAP) and its isomer α - N - oxalo - L - α, β - diaminopropionic acid (α - N - ODAP) were isolated in pure forms[3]. Ornithine and ethanolamine were also identified in the water - extract of ginseng. α - Aminoadipic acid was detected in *Panax notoginseng* for the first time.

A group of N - γ - glutamyl oligopeptides were found in the water - extract of ginseng. Oxidized glutathione(GSSG) was isolated and identified from acidic part as the main constituent of small peptide mixture. An isomer of oxidized glutathione(IGSSG) was isolated from the acidic peptides of 5th graded ginseng, its structure was suggested to be $(\gamma\text{-Glu-Gly-CysS})_2$ which was identified by amino acid analysis, N - and C - terminal analyses etc. From the neutral part, a major constituent was identified as an oxidized glutathione amide.

The materialistic nature of sleep was speculated as early as at the turn of this century. It was suggested that sleep of man or animal was affected by certain chemical substance formed in the body. A kind of sleep - inducing substance was isolated from sleep derived rabbits by Monnier and his co - workers and reported in 1977 that the structure was a nonapeptide. Since it increased δ - wave on electroencephalogram, it was then named Delta Sleep - Inducing Peptide(DSIP)[4]. From 1978 until now, our group has been studying on synthesis of DSIP and its analogues by many new coupling reagents[5]. Pappenheimer reported in 1984 that peptidoglycans as promoters of slow - wave sleep isolated and identified from human urine[6]. Very recently, Inoue reported that Sleep - Promoting Substance - B(SPS - B), a physiological sleep regulator from the brainstems of sleep - deprived rats, was identified as oxidized glutathione[7].

We have examined the physiological effects when we identified GSSG and IGSSG from ginseng. It is of interest that both GSSG and IGSSG(synthetic sample) exhibited somnogenic effects.

ISOLATION AND IDENTIFICATION

We were particularly interested in obtaining oligopeptides with molecular weights below 2,000. After many trials, ginseng oligopeptides could be separated roughly into three parts : acidic (p - 1 - m), neutral(p - 2 - m) and basic (p - 3 - m).

The following procedure was found to be convenient after many trials : the aqueous extract was deproteinized with 4% sulfosalicylic acid and followed by cation exchange chromatography to remove most of the polysaccharides and saponins. The amino acids, oligopeptides and charged substances on the column were eluted with 2% ammonia. The pH change of the eluates was very distinct and the eluate was collected in three portions : m—1, m—2 and m—3. They were lyophilized. The crude substances were gel filtrated through Sephadex G - 25 to remove low molecular weight amino acids and high molecular weihgt proteins which had not been completely precipitated by sulfosalicylic acid. From m—1, m—2 and m—3, different crude peptide mixtures were obtained and they were designated as p—1—m, p—2—m and p—3—m respectively. From the pH change of the eluate and the amino acid analysis, the three peptide mixture can roughly be classified as acidic, neutral and basic respectively and the molecular weights range from 500 to 2,000.

The three peptide mixtures were then separated by Reversed - Phase HPLC(RP - HPLC). Since the aromatic amino acid contents were very low, detection at 215nm of UV absorption had to be employed with acetonitrile as the organic mobile phase. It was found that the peptide mixtures are very hydrophilic and their retention times are very short and will be eluted almost instantaneously even with very low concentration of acetonitrile. Finally, by adjusting the pH of the eluent with trifluoroacetic acid(TFA), using TFA and acetonitrile mixture, p—1—m can be separated while p—2—m and p—3—m remain unresolvable. The reason for this difference is probably due to the acidity of p—1—m. At low pH most of the charged $-COO^-$ groups

are converted to uncharged —COOH and the polarity of the peptide molecules are decreased, the interaction of the acidic peptide and the column packing material is increased.

Peptides of p－1－m separated by RP－HPLC and eluted with an aqueous solution of TFA and acetonitrile gave five fractions designated as TFA－6, －7, －10, －12, －15. Their amino acid contents were determined.

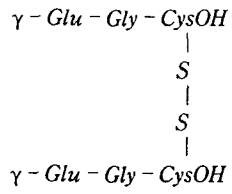
The N－terminal residues of peptides TFA －6, －7 and －15 are glutamic acid. All the peptides are proved to be γ－glutamyl instead of α－glutamyl peptides by means of DNS－Cl and Edman degradation. TFA－15 is the simplest one and its DNP derivative gives one simple product with a smaller molecular weight than the original substance on reduction with thiolethanol. By treating TFA－15 with carboxypeptidase A, releasing of glycine was observed. Its MS fragmentation is completely identical with that of oxidized glutathione(GSSG) which has been isolated for the first time from ginseng. GSSG was also detected in *Panax notoginseng*.

In order to increase the retention time of p－2－m and p－3－m, we tried several methods and finally relied on the Ion－Pair Reversed－Phase HPLC(IP－RP－HPLC) technique. We have found that tetrabutylammonium sulfate (TBAS) and camphor sulfonic acid (CSA) as a counter ion can be used successfully for the present separation.

Only one main consitituent was found in the neutral fraction, p－2－m, by IP－RP－HPLC and was designated as TBAS－14. According to analysis, the amino acids of TBAS eluate was lyophilized and oxidized with performic acid and the amino acid ratio was found to be：$CysSO_3H$：Glu：Gly＝1：1：1. The retention time of TBAS－14 is different from that of GSSG in IP－RP－HPLC. This substance was proved to be also a γ－glutamyl peptide but no amino acid was released when treated with carboxypeptidase A. On the other hand, glycine amide was isolated when treated with carboxypeptidase Y. Therefore the structure of this peptide is oxidized glutathione amide which has not been reported previously and consequently it is also a new compound. Whether the peptide is an artifact or exist *per se* in nature remains to be settled.

p—3—m was separated into distinct peaks by IP－RP－HPLC with TBAS or CSA as counter ion, but the amino acid ratios of these peaks deviated far from whole numbers and it seemed to us that they were not pure enough.

We have also studied the acidic fraction, p—1—m, isolated from the 5th graded sample, an isomer of oxidized glutathione (IGSSG), besides other γ－glutamyl peptides, was isolated probably with the following structure：



The structure of this peptide was established by TLC, HPLC, MS and synthesis.

From our studies, it appears that ginseng contains a group of γ－glutamyl peptides which caused a great deal of trouble in the determination of their sequences.

The isomeric oxidized glutathione that we isolated is of particular interest because it has never been reported since the discovery of glutathione many years ago. Whether this substance plays the same role as glutathione remains as an interesting problem for further invetigation. Studies of this group of peptides are now in progress.

SOMNOGENIC EFFECTS

The physiological effects of both GSSG and IGSSG on delta and sigma indices were evaluated after mesodiencephalic intraventricular infusion in 23 adult rabbits of either sex. Results show that both of them exerted some somnogenic effects, but they were less significant when compared to Asp^5－α－DSIP. It is beyond expection that delta－ and sigma－enhancing effects of IGSSG were more potent than that of GSSG after mesodiencephalic intraventricular infusion (50μg/rabbit, i.c.v.) in rabbits (Table 1).

Table 1. Effects of two γ－Glutamyl oligopeptides(IGSSG, GSSG, 50μg/rabbit, i.c.v.)isolated from roots of *Panax ginseng* on delta and sigma indices compared to the controls (Based on mean value of percent change between pre－and post－infusion) in rabbits

Compound	Delta index (M± SE)	Sigma index (M± SE)
Control	74± 6 (N＝7)	114± 14 (N＝7)
IGSSG	147± 9** (N＝8)	209± 14** (N＝8)
GSSG	107± 9* (N＝8)	170± 23* (N＝8)

* <0.05　** $p<0.001$

The present study demonstrates that endogenous or endogenous－mimetic substances with sleep－inducing inclusive can also be isolated from plant or herbal origin and further demonstrates that GSSG can be isolated from *Panax ginseng* root. It is especially interesting that IGSSG more potent than that of GSSG.

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