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Characteristics of Bluish Purple Pigment Produced by *Streptomyces californicus* KS-89

Beung-Ho Ryu, Young-Eh Chi, Byeong-Ho Lee*, Bub-Gyu Park and Woo-Yeol Park

Dept. of Food Science and Technology, Kyungsung University, Pusan, 608-736, Korea
*Dept. of Food and Nutrition, Dongeui University, Pusan, 614-010, Korea

Abstract

Aqueous solution pigment produced by *Streptomyces californicus* KS-89 showed a vivid bluish purple pigment and purified by silica gel column chromatography. The pigment indicated a deep purple color zone by the C.I.E. chromatic diagram, and showed UV absorption maxima at 575nm. The color intensity in aqueous solution was fairly stable in the ranges of pH 5~8 and was not affected by UV light, however, sometimes it had faded slightly by the heat. It was possible to prevent significantly by the addition of metal salt. Especially, this pigment has no mutagenicity and antitumor activity and it appears to be devoid of antibiotic activity.

Introduction

Natural pigment is essential to restore an acceptable appearance and it plays a significant part in our enjoyment of food. But, unfortunately natural pigments usually are destroyed to easy toward food processing depending on a number of factors. These factors included the fact that natural pigments are easily decolorized under the many condition of food additives, temperature, pH and sunlight, etc. However, natural pigment by microbial origin is a very useful method because this technique has a possibility of mass production and non-toxicity as compared to the economic synthetic preparation. For the purpose of use as food colors, non-toxic bluish purple pigments of microbial origin *Streptomyces californicus* KS-89 were isolated from a soil in Pusan area[1]. In a previous paper[1] optimal cultural conditions for production of bluish purple pigment already was investigated[2]. The bluish purple pigment produced by *Streptomyces califonicus* KS-89 was found to have a possibility of mass production for use of food. Therefore, the bluish pigment should be checked out to the physiological stability such as pH, heat, UV and chemicals, and also biological safety such as mutagenicity, tumor or antitumor activity, etc. This paper deals with the physical and chemical stabilities and biological properties of bluish purple pigment which produced by *Streptomyces californicus* KS-89.

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Materials and Methods

Microorganism and media

Streptomyces californicus KS-89 used in this experiment was isolated from the soil in Pusan area[1] and the GSG medium media was used[3].

Separation and purification of pigment

A fermentation broth containing bluish purple pigment was filtered with the aid of 5% active diatomaceous earth. Separation and purification procedure were carried out according to the method of Oshima *et al.*[3].

Observation of pigment by CIE system

Quantification of colour appearance, a method of quantifying the appearance of any surface color was determined by CIE(Comission International de l'Eclairage) using color and color difference meter(Nippon Denshoku CT6R−1001DT)[4]. The pigment was calculated by using the following equation :

$$X=\frac{X}{X+Y+Z},\quad Y=\frac{Y}{X+Y+Z}\text{ or }Z=\frac{Z}{X+Y+Z}$$

Assay for bluish purple pigment produced in culture broth

The amount of pigment was determined on a spectrophotometer(Shimadzu, UV-VIS-160A) by measuring its absorbance at 220~575nm of the culture filtrate. At that time, concentration of artificial food blue color No. 1, No. 2 and violet color No. 1 diluted with deionized water as 0.001% of standard colours.

Biological tests of bluish purple pigment

Determination of antibiotic activity : Antimicrobial activity was readily determined by streaking individual *Streptomyces* isolated on one side of a petri dish covering approximately one-third of the surface of the glucose peptone agar(Difco) layer. After incubation for 72~120hr at 28℃ a variety of test microorganisms were cross streaked at right angles to the border of the *Streptomyces*. The test species were compared with *Salmonella typhimurium*, ATCC 6229, *Escherichia coli* ATCC 25922, *Staphylococcus aureus*, ATCC 6538, *Proteus vulgaris*, ATCC 27519 and *Shigella flexnerii*, ATCC 9403. The cross streaked plates were reincubated at 28℃ for 48hr and examined for inhibition of the test strains[5].

Mutagenicity activity (Ames test)[6]

Bacterial strains : *Salmonella typhimurium* TA 98, TA 100, TA 1535, was kindly supplied by Prof. B.N. Ames, University of California, Barkley, CA, USA.

Induction of rat liver microsomal enzymes : The rat liver enzymes were induced with a polychlorinated biphenyl(PCB) mixture(Aroclor 1254)[6]. The induction procedure was similar to the method of Czygon et. al[7]. A single i.p. injection of Aroclor 1254(diluted to 200mg/mℓ, in corn oil) at a dosage of 500mg/kg was given to a who survived five days before sacrifice.

Preparation of liver homogenate (S−9) and S−9 mix : The microsomal preparation was made according to Garner et al.[8].

Assay of mutagenic test[6] : Mutagenicity assays were carried out using Ames strain of *Salmonella typhimurium* TA 98, TA 100 and TA 1535. Histidine(*His*$^+$) reversions were measured by standard method[6]. The standard method of assaying the mutagenic test of bluish purple pigment at the level of 500ppm and 1000ppm, which was compared with the mutagenicity of Try-p-II and aflatoxin B_1. Usually mutagens were made at 0.0012ppm of Try-p-II and 0.0012ppm of aflatoxin B_1 in dimethylsulfoxide(DMSO) as references.

Determination of antitumor activity

Animal and tumor cell : Female mice of ICR strain and Sarcoma−180 were supplied from the college of Pharmacy, Seoul National University.

Inhibition test of solid tumor growth : Tumor cells (1.0×10^6 cell/mouse) were inoculated subcontaneously into the left groin at 24 hours before the start of sample administration(7 mice to a

group). Samples were administered once a day for ten days by intraperitoneal injection, the mice were killed and the tumor were extirpated and weighed[9].

Results and Discussions

Absorption spectra of bluish purple pigment

The absorbance curves of bluish purple pigment and others from mixed bluish purple colour No. 1 and brilliant blue FCF as references were shown in Fig. 1. Visually, mixed bluish purple colour No. 1(FCF synthetic pigment) showed the most brilliant blue color, while the pigment from bluish purple pigment and blue colour No. 1 was deep blue. The absorbance curves of these three pigments showed that the maximum and peaks were 575nm and 220nm for the bluish purple pigment, 560nm and 220nm for violet color No. 1 and 620nm and 320nm for the blue color No. 1, respectively. The absorbance curves of bluish purple pigment were similiar to blue color No. 1, No. 2 and violet color No. 1. These data indicate that a high peak and low peak were 575nm and 220nm, respectively.

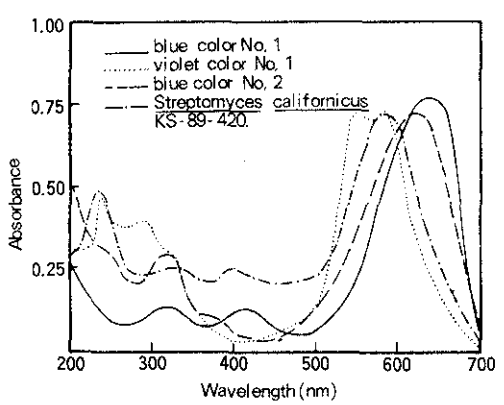

Fig. 1. Absorption spectra of bluish purple pigment produced by *Streptomyces californicus* KS−89 with comparision of artificial colours. Concentration of artificial colours diluted deionized water as 0.001% of standard colour.

We confirmed that bluish purple pigment should belong to the bluish purple pigment category.

Chromaticity of pigment by C.I.E system

C.I.E diagram is capable of quantifying the chromaticness of any colour by its dominant wavelength. X, Y, Z tristimulus values of bluish purple pigment were measured as three dimensional color space by color and color difference meter. Tristimulus values X, Y, Z were 0.3, 0.3 and 0.6, respectively. The pigment was calculated by using the following equation.

$$X=\frac{0.3}{0.3+0.3+0.6}=0.24,\quad Y=\frac{0.3}{0.3+0.3+0.6}=0.24$$

In this case, *Streptomyces californicus* KS−89−420 of the curve represents the spectrum locus, some wavelengths being indicated : the CIE diagram represents the locus of the blue and purple : the positions of the illuminants also indicated the area of bluish purple(Fig. 2). It confirmed that this pigment belongs to the bluish purple pigment category.

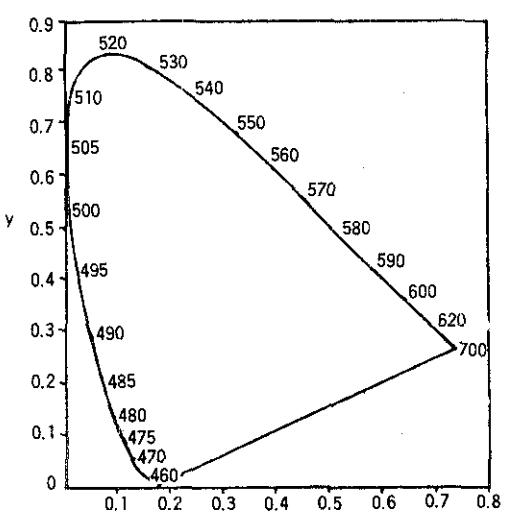

Fig. 2. The CIE chromativity diagram showing the spectrum focus/purple line and the positions of important illuminants.
X : Bluish purple colour zone produced by *Streptomyces californicus* KS−89

Effect of heat and pH of bluish purple pigment

Most foods need to be treated by heat to make food processing and cooking. At those times, natural colors occur to easy decolorization in the procedure of cooking and food processing at various temperatures. In order to maintain the food quality, sometimes natural colours are used after in processing. This experiment was carried out to design the temperature conditions of 90℃ and 120 ℃ and pH 3~9. Temperatures used in cooking and food processing can be detrimental to food pigment. Heat stability of bluish purple pigment were performed range from pH 4 to 8 to average boiling of 90℃ and 120℃ for 30, 60 and 120min, respectively. Fig. 3 showed that bluish purple pigment is stable to food processing at 30℃. However, bluish purple pigment occurred which will cause pigment marked less or changes of shade at pH 4.5 and 9.

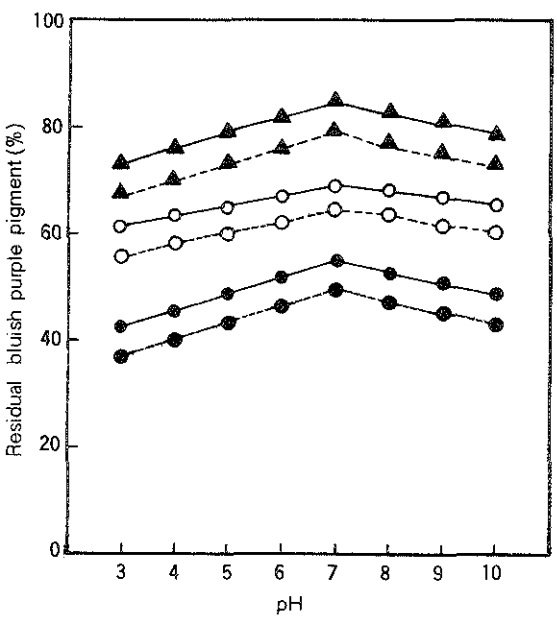

Fig. 3. Effect of pH and temperature on the stability of bluish purple pigment(90℃ : —, 120℃ ; ……).
○——○ : 30 min
●——● : 60 min
▲——▲ : 120min

Effect of metals

Natural pigment for food uses are affected by-metals and in most cases complete loss occurs eventually. This is common in some foods where metals, particularly iron, zinc, manganese, cobalt, and copper react with any compounds, with subsequent pigment loss. Bluish purple pigment was disssolved in buffer solution(pH 7.0) which measured at 1.0×10^{-2}M concentration of each metal ion such as Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Co^{2+}, Fe^{3+} and Al^{3+}. In this experiment, the mixtured ratios of bluish purple pigment and Zn^{2+} or Fe^{3+} were 2 : 1 and 3 : 1, respectively. The mixture solution was incubated at room temperature for 1 week or at 90℃ for 30 min. As shown in Fig. 4, the reaction mixture was unstable in the presence of Cu^{2+} ion, but others were relatively stable in the presence of Co^{2+}, Zn^{2+} and Co^{2+} among metal ion. In general chelate stability of metal ion were Pb>Cu>Ni>Pb>Co>Zn>Cd>Fe>Mn>Ca in order. Considerable bluish purple pigment may be replaced with Fe^{2+} in chelate compound, while it was relatively reduced in the presence at Cu^{2+}, Co^{2+} and Zn^{2+}

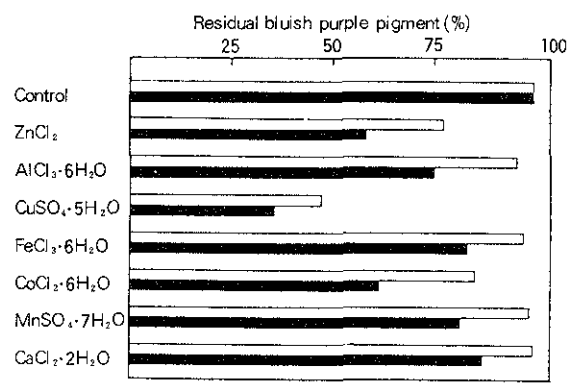

Fig. 4. Effect of metal salts on the stability of bluish purple pigment.
□ : 90℃, 30min(pH 7.0)
■ : 25℃, 1week(pH 7.0)

Antimicrobial test

Paper disk susceptibility test : The agar diffusion disk technique is the method commonly used in labolatories for measuring the susceptibility of bacteria to various antibiotic agents. As shown in Fig. 5, a filter paper disk impregnated with *Streptomyces californicus* KS−89 was placed on the agar surface previously inoculated with organism to be tested toward *E. coli.* The organism could grow up around the disk of bluish purple pigment (10 $\mu g/m\ell$) while showing distinct zones of growth inhibition around the disks such as kanamycin, tetracycline, penicillin, ampicillin and rifampicin (Fig. 5).

The Fleming ditch plate test : Fleming developed the first method for performing antibiotic susceptibility testing. GSG agar medium in the form of a plate was placed and replaced with medium containing the *Streptomyces californicus* KS−89 which are made at left angles to the plate. Multiple streak inocula of the organisms to be tested were made at right angles to the disk.

When tested at a concentration of 1~10$\mu g/m\ell$ of *Streptomyces californicus* KS−89 against 5 microorganisms by agar diffusion disk plate assay, this strain(A) gave no inhibitory activity(Fig. 6).

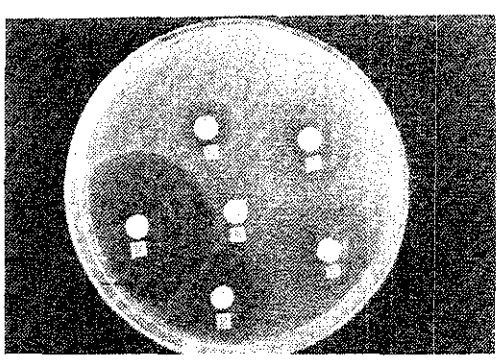

Fig. 5. Disk antibiotic susceptibility plate on which have been placed 5 disks containing a different antibiotics.
A : Bluish purple pigment, B : Kanamycin, C : Tetracyclin, D : Penicillin, E : Ampicilin, F : Rifampicin

Included in the test organism were : (B) *Salmonella typhimurium* ATCC 6229, (C) *E. coli* ATCC 25922, (D) *Staphylococcus aureus* ATCC 6538, (E) *Proteus vulgaris* ATCC 27519, (F) *Shighella flexnerii* ATCC 9403. When tested at a concentration of 1$\mu g/m\ell$ purple red pigment produced from *Streptomyces echinoruber*[13] against 19 microorganisms it appeared to be devoid of antibiotic activity. Consequently, bluish purple pigments have no antibiotic activity.

Ames mutagenicity test

Since the metabolite products of microorganisms may play a role in human carcinogenesis, their potential or actual health hazard has successfully been applied to estimate the mutagenic or antimutagenic properties of man made and naturally occuring chemicals by Ames test of *Salmonella typhimurium.*

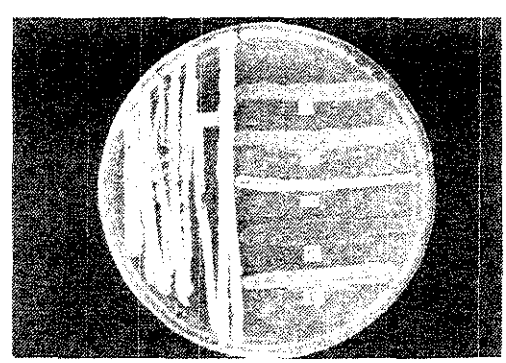

Fig. 6. Reproduction of ditch plate antibiotic susceptibility test. *Streptomyces californicus* KS−89, was placed in the ditch. In this illustration, 5 species were considered to be nonsensitive to the action of the *St. californicus* KS−89, since they could grow up to the ditch.
A : *Streptomyces californicus* KS−89
B : *Salmonella typhimurium* ATCC 6229
C : *E. coli.* ATCC 25922
D : *Staphylococus aureus,* ATCC 6538
E : *Proteus vulgaris.* ATCC 27519
F : *Shigella flexnerii,* ATCC 9403

Bluish purple pigment were contained at concentrations of 500ppm and 1000ppm per plate. Mutagenic acitivity of bluish purple pigment were carried out at 500ppm and 1000ppm giving(*His*$^+$) revertants per plate using *Salmonella typhimurium* TA 98, TA 100 and TA 1535 as indicator, with or without S−9. The mutagenic activity of Bluish purple pigment was compared with plate containing mutagens at 0.0012ppm of aflatoxin B_1 and 0.0012ppm of Try-p-II per plate. As shown in Table 1, bluish purple pigment has no mutagenic acitivity as the number of *His*$^+$ revertants.

Antitumor activity of bluish purple pigment using Sarcoma-180 bearing mice, ICR.

Antitumor activity of bluish purple pigment was attempted by using Sarcoma-180 bearing mice ICR. Antitumor activity of the pigment was not indicated *in vivo* by the occurrence and suppression of the tumor growth toward sarcoma-180 transplanted to mice. As shown in Table 2, antitumor activity of the pigment was not recognized, when it was administered by the intraperitoneal injection once a day at the dose of 10㎎, 25㎎, 50㎎, 75㎎ and 100㎎ per ㎏. The pigment appeared not to have antitumor activity at the all dose. Each sample of mice not been recognized the complete regression of tumor. These results indicated that the bluish purple pigment had no antitumor activity.

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Table 1. Mutagenic activities of bluish purple pigment which were compared with aflatoxin B_1 and Trp-p-II using *Salmonella typhimunium* strains

Sample	Concentration (ppm)	His$^+$revertants(+S9)			His$^+$revertants(−S9)		
		98	100	1535	98	100	1535
Control(DMSO)		15	87	14	12	18	8
Bluish purple pigment	500	21	22	16	0	0	3
	1000	18	20	21	5	11	8
Aflatoxin B_1	0.0012	920	1200	1010	104	105	98
Trp-p-II	0.0012	690	650	735	675	620	600

Table 2. Antitumor activity of bluish purple pigment by using Sarcoma 180 bearing mice, ICR

Sample	Tumor weight(g) (mean)	Inhibition ratio(%)	Complete repression
Control(0.9% NaCl)	2.470		
10㎎/㎏/day	2.054	16.84	0/7
25㎎/㎏/day	2.348	4.94	0/7
50㎎/㎏/day	2.085	15.89	0/7
75㎎/㎏/day	2.354	4.70	0/7
100㎎/㎏/day	2.375	3.85	0/7

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Streptomyces californicus KS-89에 의하여 생산되는 청자색 색소의 특성

류병호 · 지영애 · 이병호* · 박법규 · 박우열

경성대학교 공과대학 식품공학과
*동의대학교 가정대학 식품영양학과

요 약

천연식용 색소의 개발을 위한 하나의 방안으로 *Streptomyces californicus* KS-89에 의하여 생산되는 청자색 색소의 물리 화학적 특성을 조사하였다. 청자색 색소를 실리카겔의 칼럼 크로마토그래피에 의하여 분리하였고, C.I.E. chromatic diagram에 의하면 청자색에 속하며 UV 최대 흡수대는 575nm이였다. 이 색소의 수용액에서의 색조는 pH 5~8 범위에서는 안정하였고, 자외선에 의하여 영향을 받지 않으나, 때때로 열에 의하여 색상이 약간 흐려진다 한편 금속염에는 거의 안정하였고, 특히 이 색소는 돌연변이원성과 항암 효과는 없었으며, 또 항생 물질능도 없었음이 밝혀졌다.

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Bioavailability of Selenium

Hee-Kyung Ro

Dept. of Food Science and Nutrition, Chosun University, Kwangju, 501－759, Korea

Abstract

The bioavailability of selenium(Se) is affected by intrinsic and/or extrinsic factors. Especially, the chemical form of Se may change bioavailability. Selenomethionine, as an important naturally occurring form of Se in foods and feeds can replace methionine in a number of biochemical processes. Bioavailability assays are growth response, prevention of Se deficiency, tissue concentration of Se, Se dependent glutathione peroxidase activity and chemical balance.

Introduction

Selenium(Se) has become a very exciting topic to the academic researcher as well as to the general public since the discovery of Se as an essential nutrient[1]. The literature concerning the biochemical role of Se is extensive. Current interest is focused on the bioavailability of Se. The current knowledge concerning the efficiency of utilization of Se in the body of animals is limited.

This review will focus on bioavailability of Se related to chemical form. In addition, other factors which affect bioavailability and bioavailability assays will be briefly reviewed.

Bioavailability of Se

Definition

It is obvious that the adequacy of food to meet Se requirements depends not only on the total Se intake but also on its bioavailability[2]. Then, the practical question is, how does the body use dietary Se efficiently, since chemical analysis does not appropriately show the biologically effective level of the nutrient[3].

Fox et al.[4] defined bioavailability as follows : "a quantitative measure of the utilization of a nutrient under specified conditions to support the organism's normal structure and physiological process." Bioavailability is the proportion of a nutrient in a food used in a given biological process[5]. It is related to absorption and utilization. Utilization is the process of transport, cellular assimilation and transformation to a biologically active form[6]. Thus, the bioavailability reflects the efficiency with which ingested Se is absorbed from the gastrointestinal tract and is available for storage or further use[4].

Factors which affect the bioavailability of Se

The bioavailability of Se is affected by intrinsic factors such as the physiological status of the animal or person that consumes Se. In this category are species, genotype, age, sex, metabolic function, that is, maintenance, growth, reproduction, and lactation ; nutritional status including adaptation to dietary intake ; physiological stress, intestinal microflora and infection[6].

On the other hand, extrinsic or dietary factors affecting bioavailability are intake level of the element, chemical form, presence of promotors or inhibitors, mineral-mineral interactions, mineral-micronutrient interactions, food processing, and drugs[5－6].

Chemical forms of Se

In the periodic table Se belongs to a group of VI elements like sulfur. Se is similar to sulfur in chemical and physical properties. However, chemical reactions of Se occur much faster than those of sulfur, and biological and metabolic consequences of Se are different from those of sulfur[7]. While sulfur has a tendency to be oxidized, selenates (Se^{+6}) and selenites(Se^{+4}) are readily reduced in the body to the active selenide(Se^{-2}) form[7-8]. In the various Se-dependent enzymes, the active catalytic form of Se has been shown to occur as selenocysteine[9].

Se, which has covalent characteristics, may form organic complexes[10]. There are several organic and inorganic forms of Se which are important in nutrition(Table 1). In the diet of animals, two major chemical forms of Se are food Se and inorganic Se. Food Se from natural sources is in the form of amino acids such as selenocystine, selenocysteine, and selenomethionine[11-12]. Selenite from nutritional supplements are added as the inorganic form. In animals the existence of these different forms in feed complicates the assessment of Se bioavailability[5].

Variations exist among different chemical forms of Se in their relative ability to alleviate specific deficiency conditions. When Schwartz and Foltz[13] investigated the potency of a variety of Se compounds against liver necrosis in vitamin E deficient rats, sodium selenite, sodium selenate, selenocystine, selenocystathione and selenomethionine were almost equally protective, but natural organic Se from pig's kidney, which they designated "Factor 3", was much more effective. Elemental Se was almost inactive. Since vitamin E and certain antioxidants also play a role in protecting against the disease, this assay is not specific for Se[14]. Later, different researchers used different species. In vitamin E deficient chicks high availability values for sodium selenate and selenocystine but low values for selenomethionine, sodium selenide and elemental Se were obtained by Cantor, et al.[15]. Again, the disadvantage of this assay is that it is not specific for Se because the disease is responsive to vitamin E, too. The criteria used by Cantor, et al.[15] was prevention of exudative diathesis. However, when they used a different criterion(prevention of pancreatic degeneration), they found selenomethionine was four times as effective as either selenite or selenocystine. The difference in potency was presumed due to the ability of the pancreas of the chick to concentrate selenomethionine [16].

Osman and Latshaw[17] also reported that sodium selenite and selenocystine were almost equally effective as selenomethionine in chicks, when they used weight gain and prevention of exudative diathesis to evaluate Se bioavailability. In their study, chicks fed sodium selenite or selenocystine had a similar tissue Se content. Chicks fed selenomethionine had a lower selenium concentration in the kidneys, liver and heart than chicks fed either sodium selenite or selenocystine, but a higher concentration in the pancreas and breast muscle. Thus, the pattern of Se distribution in tissues appears to be an important factor to determine Se bioavailability though the reasons for different patterns were not well understood.

Selenomethionine may be more effective in pre-

Table 1. Some organic and inorganic forms of Se[5]

Organic forms
Selenoamino acids
Se-methionine
Se-cystine
Se-cysteine
Se-cystathionine
Se-methylselenocysteine
Inorganic forms
H_2Se, H_2SeO_3, $HeSeO_4$, CdSe

venting pancreatic degeneration in chicks because it is readily incorporated into protein and becomes concentrated in the pancreas due to the high protein turnover rate in this organ [16]. Recently Van Rij et al.[18] have demonstrated that selenomethionine retention was greater than sodium selenite in patients with very low plasma Se when they were given Se supplements in total parenteral nutrition. Thus, the different chemical forms of Se may change bioavailability. Selenomethionine may be incorporated with efficiency into tissues and retained better than sodium selenite.

Selenomethionine

Generally, plants incorporate Se into amino acids. The predominant form is selenomethionine which is an important naturally occurring form of Se in foods and feeds. Selenomethionine comprises the major portion of the Se in cereals consumed by humans[11,19]. Therefore, cereals tend to have a higher Se content than fruits and vegetables which have a low protein content[20]. The flour from hard wheat contains significantly more Se than soft wheat flour[21]. Selenomethionine, that is, methionine containing Se instead of sulfur, can replace methionine in a number of biochemical processes. In one study selenomethionine could completely replace methionine for the normal growth of a mutant of *E. coli* which had a requirement of methionine[22]. In another study B-Galactosidase produced from strains of *E. coli* had 75% of its methioninyl residues replaced by selenomethioninyl residues without affecting catalytic activity of the enzyme[23]. In addition, it was shown that selenomethionine can substitute for methionine in transmethylation reactions and in polypeptide chain initiation and synthesis in *E. coli*[24].

Recently, Sunde, et al.[25] studied the effect of dietary methionine on the bioavailability of Se from selenite and selenomethionine in rats fed a torula yeast basal diet. They found that the level of dietary methionine supplementation did not change selenite biopotency, which was quantitated by assaying liver, plasma and heart for GSH-Px. However, the biopotency of selenomethionine was increased by the dietary methionine supplementation. Thus, low dietary methionine decreased the biopotency of selenomethionine but not of selenite. It appears that selenomethionine is incorporated into general body proteins by the same pathway as methionine[26]. At suboptimal dietary levels of methionine, more selenomethionine is utilized for general protein synthesis to satisfy part of the methionine requirement and less Se is available for GSH-Px synthesis[25]. This study suggested that methionine is required for optimal utilization of plant Se as selenomethionine is presumably a major form of plant Se[11]. In addition, it was reported that vitamin B_6 deficiency impaired the increase in liver GSH-Px activity[27], erythrocyte levels of Se and GSH-Px[28] in the rat, because of interference with the conversion of selenomethionine to enzyme precursors.

Bioavailability assays

The availability of Se for absorption may or may not be a measure of its utilization. The nutritional potency of a dietary Se source is based on how well it is absorbed in addition to how well it is transformed into a biologically active form. If absorbed dietary Se is not in a biochemically active form, it must be metabolically converted into such a form[14]. Thus, Se taken as selenite or selenate must be reduced and incorporated into the selenocystine residue of GSH-Px or other selenoproteins in order to be completely utilized[6]. Methods to assess bioavailability are as follows[6] :

1. Growth response
2. Prevention of Se deficiency
3. Tissue concentrations of Se
4. Se-dependent GSH-Px activity

5. Chemical balance.

Growth response

Growth response may be used as an index when young animals can be used and Se is the only limiting nutrient[3]. The growth rate of animals fed a food source of the limiting Se is compared to that of other animals given a pure salt of Se[6]. Often, deficiency symptoms are seen in the more rapidly growing animals. If the faster growth rate results in a higher requirement, a lower estimate of bioavailability might occur[29]. Another disadvantage is that the growth response bioassay is not feasible in adult humans and cannot ethically be used with infants[6].

Prevention of deficiency

As discussed previously, species-specific criteria, such as exudative diathesis, or pancreatic degeneration in chicks and liver necrosis in rats, resulted in diverse and conflicting data[13,15,16]. Some discrepancies may result from different assay conditions and differences in the basal diet[5]. These considerations must be taken into account when the significance of animal experimental data to human nutrition is evaluated.

Tissue concentrations of Se

In animals Se is distributed in all the cells and tissues of the body. The concentrations vary with the tissue, the level and the chemical form of ingested Se[30]. When animals consume Se at their dietary requirement(0.05~0.20 ppm), Se concentrations in the liver and kidney are highest, whereas the Se content in muscle, bone and blood is lower than in the liver and kidney. The brain and nervous tissue have the lowest Se content[31].

In rats fed a torula yeast, low Se diet for 4 weeks, the Se levels in the kidney dropped from 1.0 to 0.3 ppm and in the liver from 0.7 to 0.1 ppm(fresh basis)[32]. It was also reported that the liver content of Se was more prone to the effect of dietary Se, while that of the testis and kidney was less affected by dietary Se deprivation. However, the dietary supplementation of Se as sodium selenite significantly increased the Se content of all tissues and of plasma in the same study. The differential ability of tissues to retain Se may suggest differential metabolic and functional roles of Se in different tissues[33]. Tissue Se concentrations are generally higher when Se is ingested in an organic form rather than an inorganic form[34,35] However, Se in fish meal and tuna had poor biologic availability, perhaps due to heavy metal complexing[36].

For estimating Se contents in tissues, it is necessary to treat the sample to release the Se from the matrix or chemical combination, to destroy organic matter, to dissolve Se and bring it to the required state of oxidation[37]. Digestion with a nitric/perchloric acid mixture, solvent extraction for separation of Se from interfering substances, is needed. The most popular method, the spectrofluorimetric method is based on the use of 2, 3-diaminonaphthalene(DAN) to form a fluorescent piazoselenol by complexing with Se(IV)[38]. This method has been automated for the determination of nanogram quantities of Se[39]. Spallholz et al.[40] reported a single test tube method. Neutron activation analysis is another accurate method for Se, but expensive facilities are required for its use[41]. Atomic absorption spectrometry is not as sensitive as fluorimetric methods for measuring low Se concentrations[42].

Se-dependent GSH-Px activity

GSH-Px was discovered by Mills[43] who demonstrated that it protects erythrocytes against hemoglobin oxidation and hemolysis induced in vitro by H_2O_2 or ascorbic acid. Later this enzyme was identified in rat erythrocytes as a selenoprotein by Rotruck et al.[1] and it was demonstrated that GSH-Px from ovine and bovine erythrocytes con-

tained 4g atoms of Se per mole[44]. This enzyme has since been purified from several sources including human tissues[45]. GSH-Px, with a molecular weight of approximately 80,000, consists of four identical subunits[9]. It was found that the molecular weight of GSH-Px varies with the species and the tissue[46]. In contrast to other peroxidases, this enzyme does not contain heme or flavin[47] or metals other than Se[44]. It was further demonstrated that the Se at the active site of the enzyme is in the form of selenocysteine, although the exact mechanism by which the selenoamino acid is incorporated into the protein is still unsettled[9]. GSH-Px is thought to remove H_2O_2 and a variety of organic hydroperoxides in the cell using glutathione as the source of reducing equivalents. GSH-Px may prevent · OH generation by reducing H_2O_2 to H_2O. Thus, Se-dependent GSH-Px and vitamin E complement each other to block formation of free radicals and prevent subsequent attacks on membrane phospholipids[48].

GSH-Px activtiy has been shown in various tissues, fluids, cells and subcellular fractions in animals. This activity depends on the species, tissue and Se status of the animal[49]. The highest GSH-Px activity is found in the liver and erythrocytes ; moderately high activity occurs in the heart, kidney, lung and adrenal glands. The brain, testis and lens have the lowest activity[50]. It is well established that dietary Se intake affects the tissue activity of GSH-Px. Animals maintained on Se-deficient diets show a rapid decline in tissue GSH-Px activity[51,52]. Upon Se supplementation, the Se depleted animals show a rapid increase in tissue GSH-Px activity[50-52]. In general, GSH-Px activity correlated well with total Se in whole blood or plasma in the New Zealand subjects who had marginal or deficient levels of Se intake[53,54]. However, significant correlations were not shown in individuals with adequate levels of Se intake[55]. This enzymatic method is valid only in populations with low Se intakes, since GSH-Px activity plateaus at higher Se intakes[56]. It was found that from 10 to 15% of the Se in human erythrocytes is associated with GSH-Px[57,58]. In contrast, 75 to 100% of the Se in erythrocytes of rats and sheep is associated with GSH-Px[58,59]. This finding may indicate that non-GSH-Px. Se plays a very important role in human blood[60], a factor which makes the GSH-Px assay more complicated.

Cantor et al.[16] reported that prevention of pancreatic degeneration by selenomethionine did not correlate well with pancreatic GSH-Px activity. They suggested that the biochemical function of Se in protecting the chick pancreas may not be mediated through GSH-Px. Recently Lawrence and Burk[60] discovered that Se deficient rat liver had a non Se-dependent GSH-Px activity and that this enzyme activity rises in Se deficiency. Thus, the possibility exists that biologically active forms of Se other than GSH-Px should be considered when GSH-Px activity is determined.

There are two ways to analyze GSH-Px activity. One is direct measurement of the decrease in GSH [61]. The other method involves coupling the redox cycling of GSH and GSSG to the oxidation of NADPH[62]. The latter method is more frequently used because the former method is cumbersome and insensitive. Sodium azide is used to inhibit the activity of catalase which destroys H_2O_2 · H_2O_2, t-butyl hydroperoxide, and cumene hydroperoxide can be used as the acceptor substrate. However, the Se-independent GSH-Px has peroxidase activity only against the organic hydroperoxides, so H_2O_2 usually is used as the substrate for the assay of Se-dependent GSH-Px activity. Otherwise, biased results might occur when samples contain high Se-independent enzymes. However, it was reported that enzyme assays carried out with H_2O_2 have higher blank values than those performed with organic substrates, because the non-enzymatic reaction of H_2O_2 with GSH is faster than that of

the organic substrates[57]. Another complication is that hemoglobin also catalyzes the oxidation of GSH by H_2O_2[63]. Thus, hemoglobin must be converted to methemoglobin with Drabkin's reagent before the assay for GSH-Px is performed[62]. Recently, Levander et al.[64] demonstrated that blood platelets may be useful for assessment of Se status due to their rapid turnover, high Se content and the responsiveness of platelet GSH-Px to changes in Se intake. One special concern should be given to the storage of blood samples. Butler et al.[57] found that changes in GSH-Px activity with time of storage differ for blood from various species of animals.

Chemical balance

This chemical balance technique is the most used way for determination of Se bioavailability in humans. If performed carefully and with intakes of Se near or below the minimal requirement, chemical balance may be the method of choice for evaluating Se bioavailability in adults of any species[6]. However, when intake is in excess of the requirement to maintain balance, there may be no evidence of reduced bioavailability. Furthermore, balance studies require a high degree of technical sophistication and are applicable only to small groups of subjects[65].

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생체내 세레늄의 이용율

노 희 경

조선대학교 식품영양학과

요 약

생체내 Se 이용율은 여러가지 내·외적인 요인에 의해 영향을 받는다. 그 중에서도 Se의 화학적 형태에 따라 생체내 Se 이용율이 변화된다. 식품이나 동물의 먹이에서 천연적으로 발생하는 중요한 형태는 selenomethionine으로 곡류에 있어서 Se의 주요한 부분을 포함한다. 이 selenomethionine은 생화학적인 과정에서 methionine을 대치하는 것으로 알려져 있다. 또한 생체내 Se 이용율을 판정하는 방법으로는 성장반응, 결핍예방, 조직내 Se농도, Se에 의존하는 효소 glutathione peroxidase의 활동, 화학적 평형등이 쓰인다.