Research on the Antioxidant Components of EM-X and the Mechanisms of Action

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Introduction

Infections today are caused by mechanisms different from those of past infections. And, the time until disease onset also tends to increase. These diseases definitely show a new trend different from the diseases seen in the past. One of the causes is considered to be environmental pollution together with the generation of various types of free radicals such as the activated oxygen species. To effectively eliminate these free radicals that have adverse effects on the body, a group of compounds called antioxidants are attracting a great deal of attention recently. These substances included three types; substances that directly remove free radicals in a scavenge manner, substances that promote the activities of free-radical-eliminating enzymes called SOD, and lastly SOD-like compounds. As for the anti-oxidation effect of EM-X, recent studies have confirmed clearly that it directly remove bad types of free radicals. In this presentation, the results of research on the components of EM-X and the nature of its mechanism of action, including those conducted in the past five years, will be presented. We hope to communicate these data to clinicians so that they may use this product with confidence.

Research on antioxidant capability and effects of EM-X

Fig. 1 and 2 show the research methods for the studies conducted in collaboration with the Radiation Chemistry Research Center, Shizuoka University and Nuclear Reactor Research Center, Kyoto University. The first experiment using not very strong γ rays and irradiation was conducted for a relatively long duration, and then we studied the effects of irradiation on the double helix structure of super-coil type DNA derived from *E. coli*. In this experiment, electrophoresis was conducted first. From the separated fingerprints, we input the data with an image scanner to find out the proportion of CC type DNA that maintains a double helix structure and OC type DNA that shows a broken down structure. From these areas, the proportion of residual CC type DNA was calculated. The results showed that over 90% of CC type DNA remained intact in the presence of EM-X, showing very potent antioxidation effect of EM-X.

ESR measurement and data analysis: The sample was taken out of liquid nitrogen and rapidly returned to room temperature. Then the sample was inserted in the cavity and measurement was started. The time immediately after sample was melted was taken as time O, and measurement was started 20 sec later. Seven measurements were made at intervals of 21.5 sec. The ESR spectrum was measured at each time point. The time-related changes were plotted and extrapolated to find the ESP spectral intensity at 1 sec immediately after sample was melted. This value became smaller when EM samples had greater anti-oxidation capability. The intensity of DMPO-OH after the addition of

EM material was determined compared to the DMPO-OH value before addition of EM
In the other study, free radicals were generated with very strong irradiation, with simultaneous binding of DNOP and OH. We studied how much OH was eliminated by EM-X during this process. Table 1 shows the data of residual CC type DNA as an indication of the rate of radiation protection. From the data of CC type and OC type DNA obtained in this study, it is clear that a large proportion of CC type DNA, which is the genetic filament that has not received radiation damage, remain intact.
In this study, EM-X showed a DNA cleavage rate of 8.93% at the highest, suggesting that cleavage of the double helix was almost completely suppressed. This research also showed clearly a protective effect against radiation. However, the significance of the present study lies in the data that the strong anti-oxidation capability of EM-X against OH radicals results in strong prevention against DNA cleavage.
Fig. 3 shows the results of a study irradiated with very strong γ rays using a nuclear reactor at Kyoto University. DNOP, a substance that captures OH free radicals, was used and was measured by an electron spin resonance instrument (ESR). The results clearly showed a high OH eliminating rate in the presence of EM-X. These results suggest that regardless of the SOD system, which is the intrinsic system to captures free radicals in living body. EM-X independently eliminates OH free radicals and superoxide anion radical-like free radicals. Since it is known that very strong γ rays produce large quantities of OH radicals with a short period of irradiation, these results suggest that EM-X has a very strong action in capturing OH radicals.

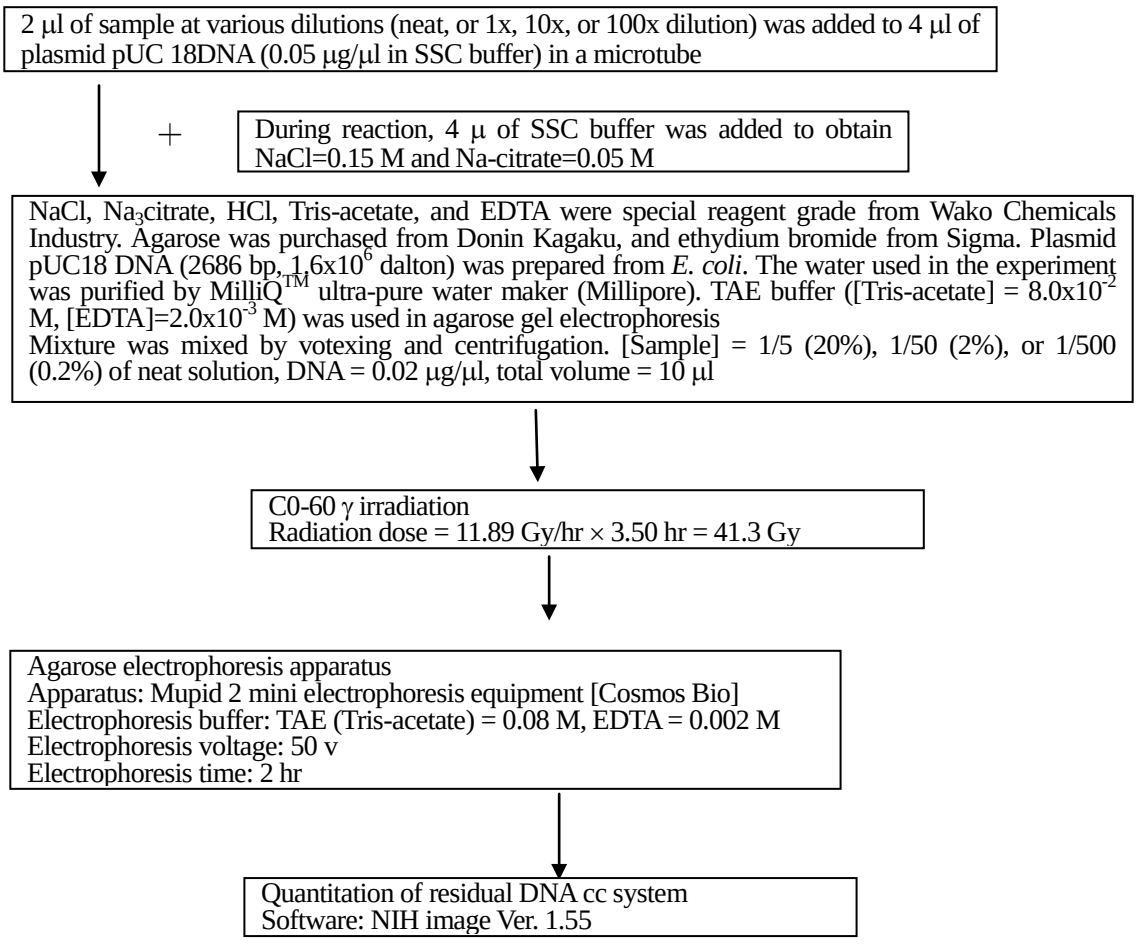


Fig. 1 Methods of Testing Hydroxy Radical Eliminating Capacity Using DNA
Research facility: Radiation Chemistry Center, Shizuoka University Department of Science

Sample: 0, 18, 36, 72 μl was dispensed in microtube.

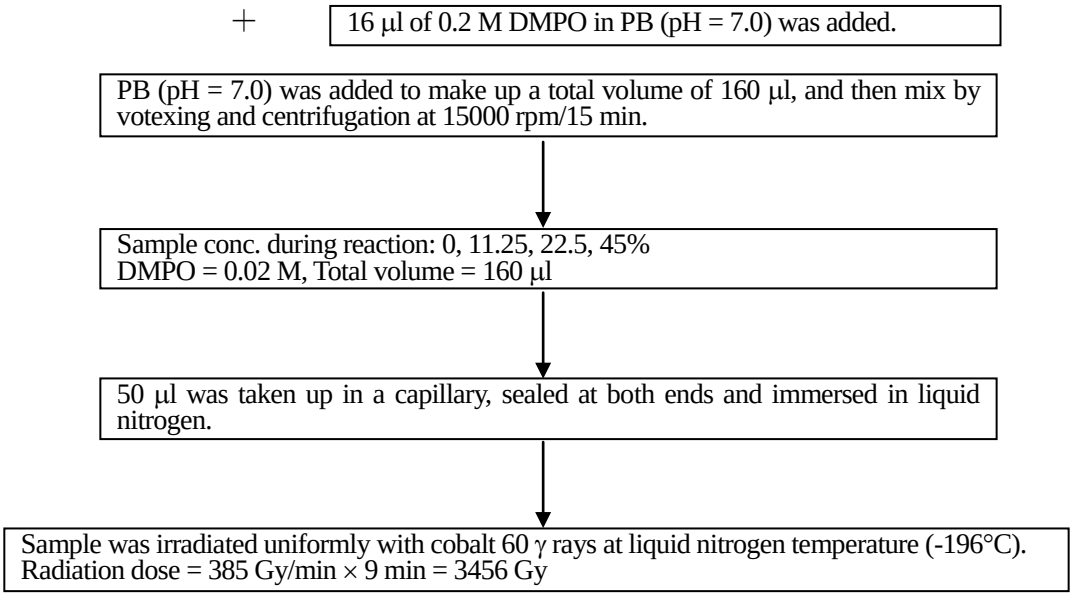


Fig. 2 Hydroxy Radical Eliminating Capacity Using ESR
Research facility: Nuclear Reactor Research Center, Kyoto University and Radiation Chemistry Center, Shizuoka University

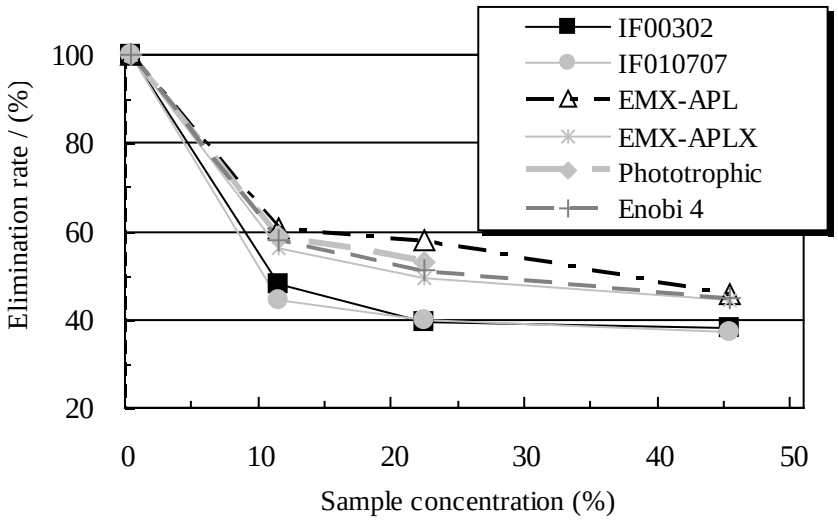
Table 1 CC Type DNA and DNA Cleavage Rate

Dilution	IFO0203 % residual cc	IFO0203 % DNA cleavage	IFO10707 % residual cc	IFO10707 % DNA cleavage
1/5	75.14	33.34	73.16	36.00
1/50	35.19	86.93	34.51	87.85
1/500	25.42	100.04	24.94	100.69
∞	25.45	100.00	25.45	100.00
Dilution	EM-ALP % residual cc	EM-XALP % DNA cleavage	EM-ALP % residual cc	EM-XALP % DNA cleavage
1/5	88.66	17.90	93.34	8.93
1/50	68.11	42.78	75.16	33.33
1/500	46.24	72.12	42.80	76.73
∞	25.45	100.00	25.45	100.00
Dilution	Phototrophic bacteria 1 % residual cc	Phototrophic bacteria 1 % DNA cleavage	Phototrophic bacteria 2 % residual cc	Phototrophic bacteria 2 % DNA cleavage
1/5	73.20	37.29	67.61	41.30
1/50	58.02	56.31	46.98	67.61
1/500	34.25	88.20	22.28	99.11
∞	25.45	100.00	21.58	100.00

Analyzed by: Radiation Chemistry Center, Shizuoka University Department of Science

Table 2 shows the OH elimination capabilities of EM-X and the component bacteria of the raw material EM-1; lactobacilli and phototrophic bacteria. This experiment used Japanese green tea as control, which has been shown to possess catechin, the substance known to have the highest antioxidation action. The results show that both lactobacilli and phototrophic bacteria possess relatively high radical eliminating capacity. From the IC50 (biological statistical system), the

radical-capturing effect of EM-X can be evaluated as being close to that of Japanese tea, which is known to be the most potent substance among foods.



Irrad. temp:	-196℃
Dose rate:	384 Gy/min
Irrad. time:	9 min
Irrad. dose:	3456 Gy
[DMPO] = 0.02 M	

Fig. 3 Evaluation of OH Elimination Capability
Date of irradiation: 8/2/2001
Date of measurement: 8/5/2001

Sample	Conc. (%)	Elimination (%)
Control	0	100
IFO0302	11.25	48.2
	22.50	39.4
	45.00	38.1
IFO10707	11.25	44.2
	22.50	39.7
	45.00	37.2
EM-X APL	11.25	60.6
	22.50	57.9
	45.00	45.7
EM-X APLX	11.25	56.3
	22.50	49.4
	45.00	44.6
Phototrophic	11.25	58.8
	22.50	53.2
Enobi 4	11.25	57.8
	22.50	51.4
	45.00	44.8

In this study, for example, IC50 = 0.1 signifies that DNA cleavage is suppressed by 50% when the reaction mixture contains 0.1 ml of the sample. This study suggests that EM-X possesses antioxidation capability close to that of Japanese tea.

Table 2 Results of IC50 (biological test evaluation system)

Sample	IC50 (dilution from neat solution) DNA	ESR
Green tea	0.0015	0.26
EM-X ALP X	0.007	0.37
EM-X ALP	0.01	-
Phototrophic bacteria (7/26 DNA test) (1)	0.04	0.3
Phototrophic bacteria (6/28 DNA test) (2)	0.08	0.1
Phototrophic bacteria (6/28 DNA test)	0.08	0.08
IFO0203	0.09	-
IFO10707	0.1	-
Enobi 5	0.12	-
Enobi 4	0.55	0.3
CO515B8 (Shiitake mushroom mycelia)	>1	-

Analyzed by: Nuclear Reactor Research Center, Kyoto University and Radiation Chemistry Center, Shizuoka University

Research on Components of EM-X

Results and Consideration

Next, the results of research on the components of EM-X will be reported. These studies were conducted from 1996 to 1997 in collaboration with the College of Science and Technology at Nihon University, Higa Laboratory at the University of the Ryukyus, Toray Research Center, Inc. and JEOL Ltd. These studies were conducted using the same methods used for detecting natural chemicals.

The results are shown in Table 3. EM-X has the characteristic that it possesses high polarity similar to water, and the components are dissolved in an aqueous state. The study used a method that solated or fractionated the components according to polarity. First, normal hexane, a solvent with low polarity was mixed with EM-X at equal volumes and shaken in a separating funnel for 30 min. The low polarity compounds in EM-X were transferred to the solvent. This procedure was repeated three times to take variance into consideration and the three solvents were mixed together. The solvent was purified with various chromatographies. The fraction of each compound at high purity was further isolated by HPLC, and single compounds were obtained. The same procedures were conducted with the high and moderate polarity fractions. We were able to isolate many fractions and crystals. The chemical structures of the substances obtained were determined by NMR to identify these substances.

From the relatively high polarity fraction, a vitamin B group was identified. In this vitamin group, B1, 2 and 12 were detected.

From the moderate polarity fraction, quercetin-3-O-glycopyranoside (quercitrin) and quercetin (flavonoid), a group of vitamin P, were detected. In addition, saponin, a triterpene glycoside with high polarity, was also detected as racemic compounds. Applying 1-butanol, a solvent with high polarity, again to this fraction, further oligosaccharides such as rhaffinose and simple monosaccharides such as glucose were also detected.

By re-extracting the low polarity fraction with moderate and high polarity solvents, vitamin E as a mixture of α, β, γ, and σ racemic forms was detected. During the process of chemical structure determination using NMR and estimation by carbon and hydrogen atomic coupling, it was judged that the racemic compounds were not composed of single compounds but contained a mixture of racemic structures. Next, we succeeded to identify this mixture using a mass analytical instrument. The low polarity fraction has the oil soluble property and so γ-olizanol and carotene do. By isolation

and purification, we detected carotene derived from the phototrophic bacteria. All these substances are antioxidants.

Table 3. Study of Components of EM-X

Low polarity compound	Medium polarity compound	High polarity compound
Vitamin E racemic bodies {α,β, γ, σ mixture}: this compound implies that vitamin E is α type.	Quercetin compounds: Quercetin-3-O-glycopyranoside, Quercetin-3-O-ramnopyranoside Campherol Campherol glucoside (2 types) (These compounds are flavonoid dyes.)	Saccharides: raffinose, glucose, and mannan Oligosaccharides and high molecular saccharides were also detected. These are relatively strong anti-oxidation compounds Trehalose, Phloricide (2-O-α-galatosylglycerol)
γ-olizanol Ubiquinone compound (many isomers of ubiquinone exist.)	Triterpene-like compounds Saponin with a 20s-protopnexatriol structure (from the sugar binding site, determined to be zinsenoside-R0 by NMP and MS.)	NAD (nicotinamide adenine denucleotide) and FAD (flavine adenine denucleotide): substances that promote differentiation and assimilation of life. Cyanocobalamin with relatively large MW and its isomers were also detected. (These were novel compounds.)
Carotene dyes and Phototrophic bacteria-derived dyes including lycopene: strong antioxidation effects	Vitamin C (reductive form is more abundant than oxidative form)	Inositol, L-aspagine acid L-alanine Analytical results also showed other amino acids.
Trace lipids: Phosphophatidyl choline Glycerin ester glucoside	Nicotinic acid, Coenzymes (derived from nicotinic acid), NMA, etc.	Other polyphenols

Study facilities: University of the Ryukyus, Nihon University, Toray Research Center, Inc. and JEOL Ltd., 1998-1999

In this study, other chemical compounds were also detected, but the major substances for which chemical structures have been identified are shown.

Table 4 shows the substances contained in the high polarity fraction. These substances are derived from coenzymes such as nicotinamide adenine dinucleotide (NAD) and flavine adenine dinucleotide (FAD).

As shown here, compounds have been isolated and purified from the high polarity fraction of EM-X and their structures had been identified. Many of the compounds have already been proved to be antioxidants, but substances with unknown functions were also isolated.

Table 5 shows amino acid groups also detected in the high polarity fraction.
All the data reported above were obtained in the earlier research of organic compounds in EM-X.

Table 4 Compounds Isolated and Purified from High Polarity Fraction

Table 5 Results of Amino Acid Qualitative Analysis

1. Cyanocobalamin
2. Constitutional isomer of Cyanocobalamin
3. Triterpenoid compound
4. Saponigen (aglycon : saccharide unbound nuclear substance of saponin)
5. β-sitosterol
6. Constitutional isomer of β-sitosterol
7. Polysaccharide (glucomannan) suggested to bind with 1-6-glucoside derived from yeasts
8. Polysaccharide compound
9. Polyphenols
10. Phenylpropatides
11. 2,4-methylenechlorotanol ferulic ester
12. Metallic organic ismoers

Amino acid
Cystine
Alginine
Asparaginic acid
Glutamic acid
Glycine

Table 6 shows the mineral contents of EM-X. These are the first data from Fluorescent X-ray analysis and plasma chemiluminescence analytical device of Toray Research Center, Inc. The aim of this study was to investigate what kinds of minerals are present in EM-X. Forty kinds of minerals were detected at ng level. These data confirmed that EM-X contained many types of minerals. The values in Table 6 are shown in ng.

Table 6 Analysis of Mineral Components in EM-X

LI	B	Na	Mg	Al	Si	P	K	Ca	Ti
2.5	1000	2000	2000	10	10	7000	5000	2000	1
V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge
5	5	200	50	5	5	100	5	30	30
Se	Sr	Z r	Nb	Mo	Ag	Cd	In	Sh	Sb
25	10	10	3	5	7.5	1	40	15	10
Te	Ba	La	Ce	Ta	W	Pt	Au	Pb	Bi
25	5	7.5	30	30	250	10	7.5	10	20

Analyzed by: Toray Research Center, Inc.
Qualitative and quantitative results (ng/ml)

Table 7 shows the analytical results of the components of EM-X conducted in collaboration with Institute of Food Hygiene, Japan Food Hygiene Association, according to methods for food analysis. The results showed a calorie level of 7 kcal, water content of 98.1 g, protein of 0.1 g, carbohydrate of 1.7 g, ash content of 0.1 g, and sodium of 14 mg all per 100 g of sample. These data are the nutritional component analytical items for 100 g of EM-X.

Table 7 Results of Nutritional Analysis of EM-X

Test item	Test results	Analytical method
Calorie	7 kcal	
Water content	98.1 g	Vacuum heat desiccation
Protein	0.1 g	Kjeldahl Method
Lipid	0 g	Ether extraction
Dietary fiber	0 g	Prosky method
Ash content	0.1 g	Ashing method
Sodium	14 mg	Atomic absorption method
Iron	0.8 ppm	Atomic absorption method
Zinc	0.2 ppm	Atomic absorption method
Copper	Undetectable	Atomic absorption method
Iodine	Undetectable	HPLC
Serine	Undetectable	Spectrophotofluorimetry

Analyzed by: Japanese Food Hygiene Association Research Institute

Remark:
Calorie = protein x 4 + lipid x 9 + carbohydrate x 4
Carbohydrate = 100 – (water + protein + lipid + ash + dietary fiber)

Table 8 shows the results of component analysis for the items such as calcium, phosphorus, potassium, magnesium, retinol, β-carotene, vitamin A effect, vitamin B complex (B1, B2, B6, and B12), vitamin C, vitamin D, vitamin K, and folic acid. The analytical methods were according to the JIS or JAS standards. Other analyses included iron, zinc, copper, iodine, selenium, and γ-olizanol. The results are as shown in the Table 8. Since these methods were based on food components, the special carotene derived from phototrophic bacteria was technically difficult to analyze. Selenium was detected qualitatively but the concentration was below the detection limit for food, so it is recorded as not detected. For mineral analysis, we have a different set of data from Toray Research Center, which will be presented later.

Table 9 shows the results of qualitative and quantitative analyses according to food standards conducted at Toray Research Center. These results were from ICP-MS and atomic absorption analysis. The analytical results are highly reliable. In this set of data, selenium and other elements that were not clearly shown in previous analyses were clearly detected here. These minerals can be judged to have great effect on the antioxidant action. The number of minerals detected this time was only approximately half of the 40 types analyzed previously. This is because this analysis was based on food analytical methods. It is noteworthy that most of these minerals are considered to be very important for the biological activities of living organisms.

Table 8. Component Analysis of EM-X

Analytical item	Analytical result	Analytical method
Calcium	1mg	Atomic absorption
Phosphorus	4mg	Molybdovanadate method
Potassium	16mg	Atomic absorption
Magnesium	2 mg	Atomic absorption
Retinol	0μg	HPLC method
β-carotene	0μg	HPLC method
Vitamin A effect	1.0 IU	
Vitamin B1	1.44 mg	HPLC method
Vitamin B2	0.54 mg	HPLC method
Vitamin B6	3.94 mg	Microbiological assay
Vitamin B12	59 μg	Microbiological assay
Vitamin C	0 mg	HPLC method
Vitamin D	50 IU	HPLC method
Vitamin E	7.3 mg	HPLC method
Vitamin K	0 μg	HPLC method
Folic acid	2.77 μg	Microbiological assay

Analyzed by: Japanese Food Hygiene Association Research Institute
(Values for 100 g of sample)
Remark:
Calorie = protein x 4 + lipid x 9 + carbohydrate x 4
Carbohydrate = 100 – (water + protein + lipid + ash + dietary fiber)

Table 9. Detailed Analytical Results of Mineral Contents of EM-X

Element	Analytical result	Element	Analytical result	Element	Analytical result

Li	+	Y	-	Ho	-
Be	-	Zr	-	Er	-
B	++	Nb	-	Tm	-
Ma	++++	Mo	+	Yb	-
Mg	++++	Ru	-	Lu	-
Al	++	Rh	-	Hf	-
Si	+++	Pb	-	Ta	-
K	++++	Ag	-	W	-
Ca	++++	Cd	-	Re	-
Sc	-	In	-	Ir	-
Ti	-	Sn	-	Pt	-
V	-	Sb	-	Au	-
Cr	+	Te	-	Hg	-
Mn	+++	I	+++	Ti	-
Fe	++	Cs	-	Pb	-
Co	-	Ba	+	Bi	-
Ni	+	La	-	Th	-
Cu	+	Ce	-	U	-
Zn	++	Pr	-	P	+++
Ga	-	Nd	-		
Ge	-	Sm	-		
As	+	Eu	-		
Se	-	Gd	-		
Rb	+++	Tb	-		
Sr	+++	Dy	-		

Mg	81	Zn	0.11
P	120	Na	46
Ca	8.9	K	190
Fe	0.06	I	0.71
Cu	0.02	Se	0.001

Analyzed by: Toray Research Center, Inc.
Quantitative results: values in μ b/ml
Qualitative test:

Fig. 4 attempts to explain the antioxidant action of the minerals. They are the most important content for EM-X. The fundamental nature of antioxidant effect has been presumed to be the mineral complex. As one of the facts supporting this, Sato, et al. have proved that the suppression of rusting is related to metal complex, and a patent has been obtained based on this finding. In the living body, also metal complexes are known to be related to the inhibition of oxidation.

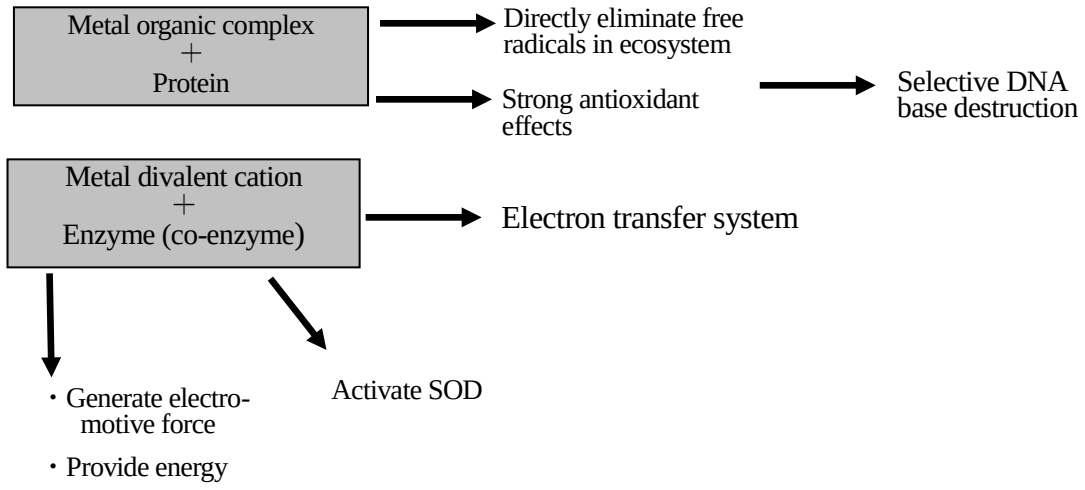


Fig. 4 Antioxidant Effect of Metals

Our hypothesis is as follows. In the case that blood flow in the blood vessels is impaired by some reason, when the flow is resumed, a large quantity of SOD is generated. This triggers the production of reactive-oxygen species with even higher activities, which destroy the cell membrane. In this case, administration of SOD beforehand has been reported to suppress the impairment. However, since SOD is a protein, its direct administration into the body will result in the compound being decomposed by proteolytic enzymes. To avoid the action of proteolytic enzymes, various methods have been used to preserve SOD activity such as chemical treatment or enclosing in lipid membranes. Since SOD is an enzyme containing metal, small metal complexes that possess the same action as SOD have been proposed. These small metallo-complexes have been confirmed to have the ability to cleave superoxide anions into oxygen molecule and hydrogen peroxide. One portion of the superoxide anion is oxidized into oxygen molecule while the other portion is reduced to hydrogen peroxide. By receiving the redox electron, the metallo-complexes are expected to become a low-oxidized state. The metal ion that now exists in a low-oxidized state further reacts with superoxide anions. It returns to its original oxidized state by donating one electron to the superoxide anions which turn to superoxide anions with extra electron. Within the reaction system, the superoxide anions with extra electron react with hydrogen anions and become hydrogen peroxide. These reactions repeat catalytically as long as superoxide anions are present. In other words, the superoxide anions are finally eliminated.
The latest research on components has been presented in the 2001 research report, where amino acid analysis was conducted in detail. Table 10 shows a portion of the component analysis. The analytical results for amino acids are similar to the last analysis, except that the quantitative values shown are higher than the previous values. This is because the EM-X sample was concentrated 4 times, thus increasing the analytical sensitivity.
Table 11 shows the analysis of amino acids, showing high contents of amino acids derived from yeast nucleic acid and components derived from seaweed.

The amino acid content is high, especially, proline is found to be the predominant component in heated EM-X products. This is considered to be component derived from DNA as a result of self-digestion of yeasts. Furthermore, alginine is thought to be a substance derived from seaweed. In 18 kinds of nutrients, almost all essential amino acids are present. This factor, in addition to the antioxidation capability, is highly significant.

From the research of component analysis and mechanisms, new knowledge was found, especially from the detection of amino acids. As already shown in basic research in the past, new research from the viewpoint of nutrition-improving functional foods confirmed that EM-X "contains a high metal content" and "contains a relatively large quantities of vitamins and physiologically active substances such as FAD". Furthermore, the new research also demonstrated that these compounds are all antioxidants. It is perceivable that these diverse components interact synergistically to exhibit strong antioxidant actions.

Table 10. Analysis of Antioxidant Components of EM-X
(Values for 100 g of sample)

Test item	Test result	Test method
Carotene	0.34 mg	JIS556
Retinol	0.21 μg	JIS556
Vitamin A effect	0 IU	JIS556
Vitamin B1	0.70 mg	JIS556
Vitamin B2	0.54 mg	JIS556
Vitamin B6	1.74 mg	JIS556
Vitamin 12	0.2 μg	JIS556
Vitamin C	1.6 mg	JIS556
Vitamin D	0.002 μg	JIS556
Vitamin E	6.1 mg	JIS556
Vitamin K	0.004 mg	JIS556
γ-oryzanol	ND	JIS556
Lycopene	0 mg	JIS556
Fructose	0.6 mg	JIS556
Glucose	0.4 mg	JIS556
Niacin	5.2 mg	JIS556
Folic acid	23μg	JIS556
Inositol	0 mg	JIS556

Table 11. Results of Amino Acid Quantitative Analysis

Mg/100 g	Heat-treated EM-X	Non-heat-treated EM-X	Refined EM-X
Isoleucine	20	8	9
Leucine	30	11	13
Lysine	44	17	19
Methionine	2	1	1
Cystine	1	1	1
Phenylalanine	21	8	10
Tyrosine	35	14	16
Threonine	29	11	13
Tryptophan	15	5	7
Valine	22	9	10
Histidine	14	5	6
Alginine	22	9	10
Alanine	19	8	9
Asparaginic acid	4	1	1
Glutamic acid	15	8	9
Glycine	50	19	22
Proline	76	28	33
Serine	9	4	4
Asparagines	-	-	-
Glutamine	-	-	-

Analytical methods: Amino acid autoanalyzer
Analyzed by: Japanese Food Hygiene Association Research Institute

However, the research conducted in Shizuoka University strongly suggests that besides the antioxidant effect mentioned above, EM-X also has other roles. For example, when hydrogen peroxide, one type of activated oxygen species, is synthesized in the body, it reacts with trace metals such as iron ion or its complex to generate hydroxyl radicals. Since hydroxyl radical possesses a strong oxidizing capability for various substances, it might "inhibit the proliferation of cancer cells or kill the cancer cells". The metal-hydrogen peroxide complex/hydroxyl radicals are thought to slip between the base pairs of the double strands of DNA and bind strongly there, a process called intercalation. Then the metallic ion portion reacts with oxygen molecule to produce activated oxygen species, cleaving the DNA. Depending on the structure of the complex, "it may occupy the space of the gap formed by twisting of the DNA double strands".

In Fig. 5 the presumed structure of the complex is shown as reference. This kind of complex is capable of entering the space of this gap here of the DNA double strands. In the DNA structure, there are alternating large gaps and small gaps. Some complexes may wind round the gap, while complexes with different structures may fit into the gap and bind with the DNA. The metallic ion of each complex then reacts with oxygen molecule, forming activated oxygen species to cleave the DNA. Or, they may form grouping to change the overall structure of the DNA, rendering the DNA of cancer cells impossible to replicate. EM-X has been proved to contain metal complexes. We are in the process of determining the structural formulae of these complexes.

The mechanism of the antioxidant effect of EM-X was more clearly elucidated in the present radiation chemical research. Of course, based on the research results so far, a clear antioxidant effect from nutritional elements is also essential for EM-X.

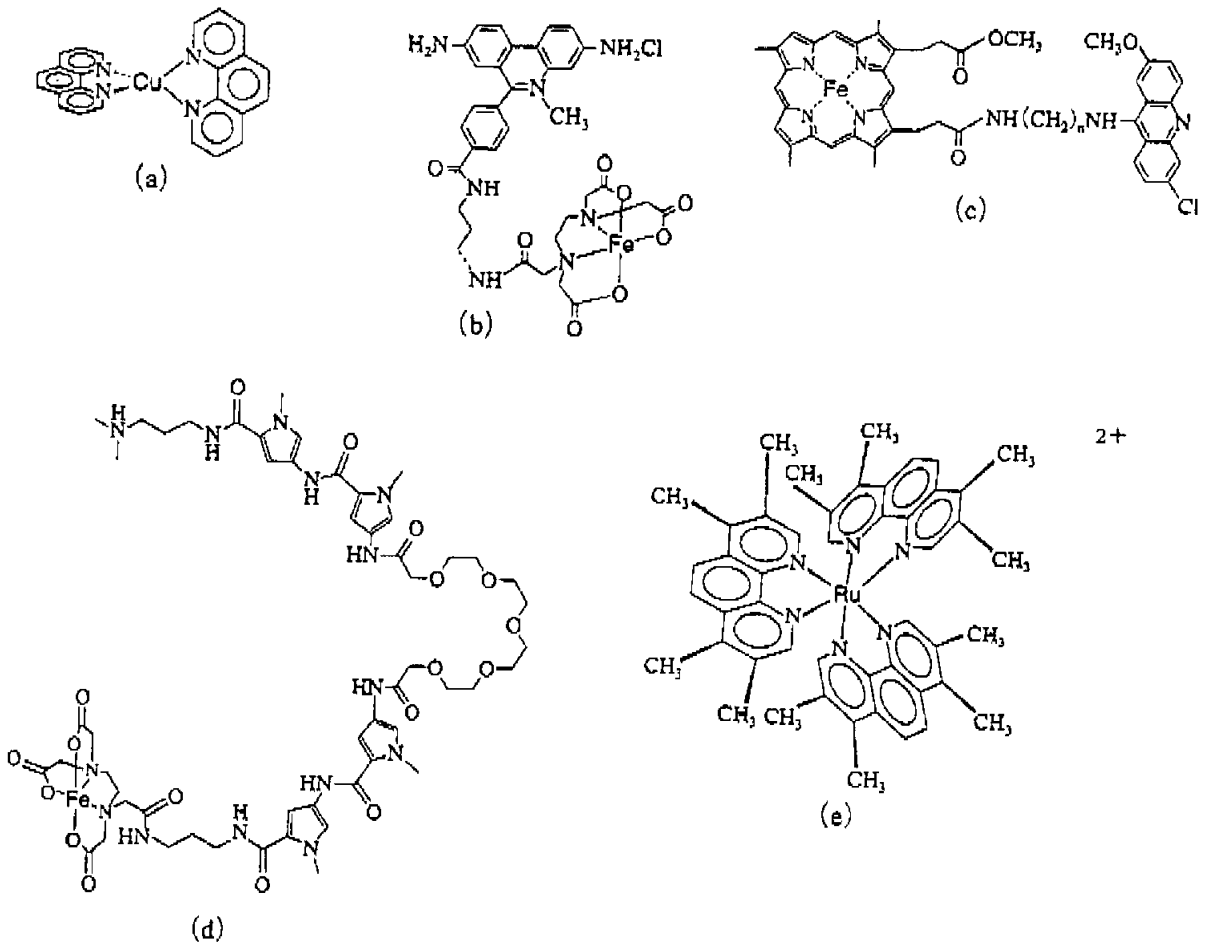


Fig. 5. Estimated Structures of Oxygen-activating Metal Complexes of EM-X

Conclusion

The research on EM-X has continued from 1997 to 2001. In this presentation, a part of the results are summarized. The characteristics of EM-X can be summarized as shown below. In addition, our research can also be categorized into two parts.

A. Antioxidant action

1. The antioxidants in EMX are substances derived from the raw materials of plants, seaweed and rice bran; substances derived from the fermentation of various microorganisms (EM-1); and substances derived from secretions of the microorganisms.

2. The components derived from plants include saponin (a glucoside of triterpene), flavonoid, and γ-olizanol.

3. Besides the above substances, vitamins were detected.

The above substances directly eliminate free radicals, and promote the action of free radical elimination of SOD by donating electrons to SOD.

B. New research findings

1. In radiation experiments in which free radicals are generated in a large amount, when DNA derived from *E. coli* was irradiated by strong γ rays generated from a nuclear reactor, the residual non-destroyed gene signals far exceeded the destroyed gene signals in the presence of EM-X, and gene destruction was inhibited by 90%.

2. EM-X was potent in removing OH radicals, clearly showing antioxidant capability as a scavenger.
3. In EM-X, the minerals consist of a relatively large number of complex forms. These mineral complexes are associated with selective formation of strong free radicals against cancer cells, which results in damage of cancer cells. This was proved by the experiment of irradiating *E. coli* super-coil type DNA with relatively weak γ rays, in which a large quantity of CC type DNA remained intact (gene destruction inhibiting gene). If irradiation is conducted under the presence of large quantities of soluble metals, a large quantity of free radicals is produced, resulting in an opposite effect. However, DNA is not destroyed in EM-X treated group. This may be due to the following; even in the presence of metallic ions, metallic organic complexes that are more potent in action bind with specific sites of the DNA, or there is selective action from protein binding.
Finally, we are now examining the feasibility to generate the data of a clinical trial entitled "Metabolism and safety of healthy subjects given EM-X" within 2 years.