Antimutagenesis and Anticarcinogenesis Mechanisms II

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DEDICATION
IN MEMORY OF

Dr. Tsuneo Kada

Dr. Alexander Hollaender

With respect and affection, the editors dedicate this volume to Dr. Tsuneo Kada and Dr. Alexander Hollaender, each of whom played a significant and important role in the establishment and continuation of these Conferences.

Tsuneo Kada was born in Japan, where he lived and worked throughout most of his scientific career. He received his undergraduate degree from the University of Tokyo and his doctorate in Natural Sciences from the University of Paris in 1963 for studies on the mechanism of mutation induction by X-rays and ultraviolet light in E. coli. He also studied in the United States, and was a frequent participant in international conferences and meetings. Dr. Kada was a pioneer in the area of studies on antimutagenesis, and it was his suggestion that led to the first International Conference on Mechanisms of Antimutagenesis and Anticarcinogenesis, for which he served as co-organizer and in which he participated vigorously. He will long be remembered for his numerous scientific contributions, including the development of the widely used "rec assay," and for his infectious enthusiasm which stimulated his colleagues and students. He was Professor and Head of the Department of Molecular Genetics at the National Institute of Genetics in Mishima, leading the planning for this Conference at the time of his untimely death in November of 1986.

"Alex" Hollaender was born in Germany, but in 1921 moved to the United States, where he pursued his educational and scientific careers. He received all of his degrees from the University of Wisconsin, where he completed his Ph.D. in 1931. He did pioneering experimental research in radiation biology, and was a driving force behind the organization of the Radiation Research Society and the Environmental Mutagen Society, and their international counterparts. He was also instrumental in founding the Biology Division of the Oak Ridge National Laboratory and served as

its Director for 20 years. From 1973 until his death in 1986, he operated the Council for Research Planning in Biological Sciences in Washington, DC, which played a strong advocacy role for science and scientific communication. He was a member of the National Academy of Sciences and holder of innumerable prestigious awards. He was enthusiastic, persistent, helpful, and loved and respected. At the time of his death in December of 1986, he was deeply involved in the planning for this Conference.

For their pioneering scientific contributions, and for their key roles in stimulating and organizing these Conferences, we dedicate this volume to our departed friends and colleagues.

Yukiaki Kuroda
Delbert M. Shankel
Michael D. Waters

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Antimutagenesis and anticarcinogenesis and the intricate relationships between them are of clear significance in cancer, hereditary illnesses, and perhaps even in aging. The elaboration of the mechanisms involved in these processes can confer substantial benefits on this and future generations. It is our hope that bringing together the basic, clinical, and applied scientists who generously shared their data and their thoughts, both formally and informally, throughout this Conference will increase our understanding and lead to those future benefits.

The success of a conference depends upon the combined efforts, skills, and support of many individuals and organizations. Listed below you will find the membership of the International Advisory Board, the Japanese Advisory Board, the Organizing Committee, and the Local Committee. Their contributions were all important. Also listed are those local and federal agencies and companies in Japan and the United States which contributed financial support for the meeting. Without their support, the Conference could not have been successful; and we are deeply grateful for that support. Less obvious, but also important, were the contributions of the excellent secretarial and logistic support provided by the Conference Secretariat Staff at the National Institute of Genetics and by the staff of the Ohito Hotel at the conference site.

We are also indebted, of course, to each of the major speakers for their excellent presentations, and to all of those who presented short oral papers or posters and participated in the intellectual excitement of the meeting.

We are doubly grateful to those who have prepared their manuscripts for inclusion in these proceedings. The final success of any meeting depends upon the quality of the speakers and papers--and that quality was very high at this meeting, as evidenced by these papers. We express also our deep gratitude to Ms. Claire Wilson and the staff of Claire Wilson & Associates in Washington, DC, for technical editing and typing, and to the staff at Plenum Press who joined with Ms. Wilson to assure that these Proceedings could be published rapidly and efficiently.

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In addition to Drs. Y. Kuroda (Chair) and D.M. Shankel (Co-chair), the other members of the Organizing Committee were Drs. Y. Shirasu (also Co-chair), Y. Sadaie (Secretary), H. Tezuka (Treasurer), and I. Tomita (Program).

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To all of the above, and to all of those who participated, we express our sincere thanks.

Yukiaki Kuroda
Delbert M. Shankel
Michael D. Waters

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ANTIMUTAGENESIS STUDIES IN JAPAN

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INTRODUCTION

Studies on antimutagenic factors were initially carried out in the 1950s in the field of microbial genetics [for review, see Clarke and Shankel (3)]. During the past ten years, a wide variety of chemical mutagens and carcinogens has been detected in foods, medicines, cosmetics, insecticides, and even in the atmosphere and water which we utilize daily. Some mutagens act directly on cells to produce mutations, and others act following their modification by other factors. Some of these indirect mutagens are metabolically activated by enzymes in organs or tissues, and others may be inactivated and inhibited by some dietary foods and by components of our cells.

In Japan, Tsuneo Kada (10,20), a pioneer in antimutagenesis studies, found effective factors in vegetables and fruits which inactivate the mutagenic action of amino acid pyrolysis products. He has proposed a new word, "desmutagens," for factors which act directly on mutagens or their precursors and inactivate them (20). It has been proposed that other factors which act on the processes of mutagenesis or repair DNA damage to result in decreasing mutation frequency should be called "bio-antimutagens" (11). Figure 1 indicates the actions of desmutagens and bio-antimutagens in the process of mutation induction.

So we can divide antimutagenesis into two different processes: desmutagenesis and bio-antimutagenesis. This article reviews antimutagenesis studies in Japan.

DESMUTAGENESIS

The mechanisms of desmutagenesis involve the following processes:

1. Chemical inactivation of mutagens.

2. Enzymatic inactivation of mutagens.

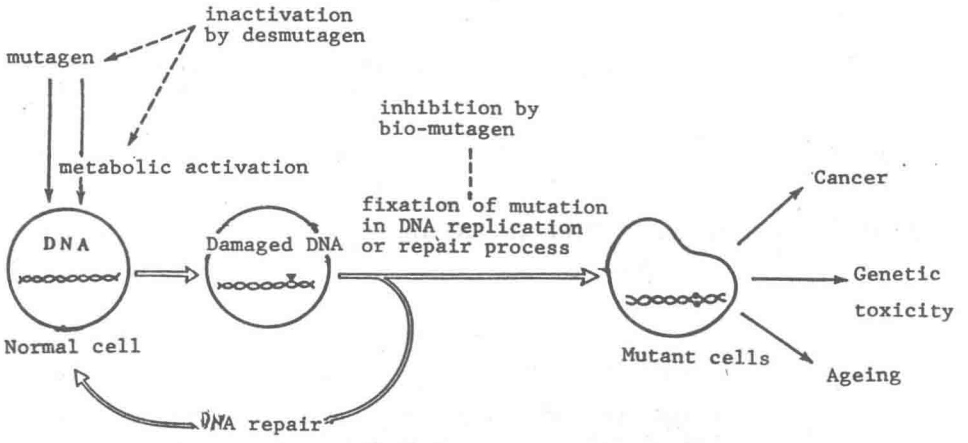

Fig. 1. Schematic representation showing the process of antimutagenesis (11).

3. Inhibition of metabolic activation of promutagens.

4. Inactivation of activated mutagens, including scavenging.

Chemical Inactivation of Mutagens

Most mutagens are chemically-active substances which act not only on DNA but also on proteins and enzymes. This suggests that mutagens may frequently be inactivated by other factors. For example, some mutagenic pesticides, such as captan, captafol, folpet, and 2,4-dinitrophenyl thiocyanate (NBT), are extremely sensitive to compounds containing the sulfhydryl group, such as cysteine, rat-liver homogenate, and blood (21). The numbers of revertants induced by these pesticides in Escherichia coli and Salmonella typhimurium were reduced by cysteine, liver homogenate, or blood. These characteristics should be considered in any explanation of the lack of carcinogenicity in the chronic feeding studies on captan, captafol, and folpet. Various factors which inactivate some direct mutagens have been extensively surveyed by Onitsuka et al. (32), as shown in Tab. 1. It is known that some food additives react with each other and produce new mutagens. Sorbic acid (2,4-hexadienoic acid) is a widely used food preservative. Nitrite is also a food additive used as a preservative of meat products, and often co-exists with sorbic acid. These two food additives, sorbic acid and sodium nitrite, can react to produce a mutagenic nitroso-compound (Y substance). It was found that vitamin C has the ability to react with Y substance and produce a nonmutagenic compound (33). Sodium nitrite also reacts with dimethylamine contained in various foods and produces a strong carcinogen, nitrosoamine. Proline has a competitive inhibitory action with the reaction between sodium nitrite and dimethylamine. Proline reacts with sodium nitrite and produces noncarcinogenic nitrosoproline, which is excreted to the outside of the body by urine (44).

The pyrolysis products of amino acids and of certain foods have a strong mutagenic activity (22,42). Negishi and Hayatsu (23) found that cysteine and its derivatives have an enhancing effect on the mutagenicity

Tab. 1. Inactivating factors of some chemical mutagens (32).

Factors \ Mutagens	Dexon	Captan	MC	4NQO	MNNG
Control	-	-	-	-	-
Vitamin C	++	++	++	-	++
D-Erythrobin	±	++	++	-	++
BHT	-	+	-	±	-
Gallic acid	++	±	++	-	-
n-Propyl gallate	-	±	+	-	-
Vitamin E	-	+	-	-	-
Glutathione	++	++	++	-	++
L-Cysteine	++	++	++	+	++
Cysteamine	+	++	+	+	++
Thiourea	-	++	-	-	-
AET	-	++	++	-	-

Test system : Rec-assay

of tryptophan pyrolysis products, Trp-P-1 and Trp-P-2. On the contrary, Arimoto et al. (1) in the same group demonstrated that hemin and other biological pyrrole pigments have an inhibitory effect on the mutagenicity of tryptophan and glutamic acid pyrolysis products. Hemin, biliverdin, and chlorophyllin produced inhibitions of all six mutagens tested, and protoporphyrin inhibited three of them (Tab. 2). Hemin was the most effective among these pigments. It was suggested that hemin may interact with the metabolically-activated form of amino acid pyrolysis products and as a result may inhibit mutagenicity.

Tab. 2. Inactivation of the mutagenicity of amino acid pyrolysis products by pyrrole pigments (1).

Mutagen	Dose of pigments required for 50% inhibition (nmole/plate) Hemin	Chlorophyllin	Biliverdin	Protoporphyrin
Trp-P-1	3	100	200	50
Trp-P-2	20	200	500	100
Glu-P-1	75	100	300	(-)
Glu-P-2	40	150	200	(-)
Amino-α-carboline	30	150	500	(-)
Aminomethyl-α-carboline	25	200	500	50

Test system : TA98 + S9 Mix

Enzymatic Inactivation of Mutagens

Environmental mutagens, especially natural mutagens, are often inactivated by various enzymes contained in the organs and tissues. Kada et al. (9) found that juices of vegetables such as cabbage, turnip, radish, or ginger contained antimutagenic factor(s) which acted on tryptophan pyrolysis products in Salmonella TA98 with rat liver homogenate activating mixture (S-9 mix) (Tab. 3). This antimutagenic effect was abolished by prior heating of the juice at 100°C. This indicates that the antimutagenic factor(s) may be a desmutagen(s) and is a heat-sensitive substance(s).

The desmutagenic factors of cabbage juice were purified by procedures shown in Tab. 4 (4-6). The mutagenicities of Trp-P-1 and Trp-P-2 were abolished by this factor. Mutagenic activities of ethidium bromide and 2-aminoanthracene (2AA) were also susceptible to the factor. However, the factor had no effect on the mutagenicity of ICR-170 or furyl-furamide (AF2). The purified factor had a molecular weight of 43,000 and had 54.2 μg of sugar per mg protein. The factor exhibited a hemoprotein-like absorption spectrum with a Soret band at 404 nm, and α and β bands at 640 nm and 497 nm, respectively. The Soret band shift resulting from treatment with sodium hydrosulfite and cyanide indicated that the factor had very similar properties to horseradish peroxidase with NADPH-oxidase activity. The factor had both peroxidase and NADPH-oxidase activities. This suggests that the desmutagenic activity of vegetable juice may be due to the inactivation of amino acid pyrolysis products by these enzymes.

Myeloperoxidase that was extracted from human promyelocytic leukemia HL-60 cells also has the ability to degradate pyrolysis products of tryptophan, glutamic acid, and globulin (46). This is another example of enzymatic inactivation of mutagens, which may be a general phenomenon. Enzymatic detoxification in the liver has been extensively examined during studies on drug metabolism.

Inhibition of Metabolic Activation

It is known that many procarcinogens are changed into ultimate carcinogens by metabolic activation in cells. In the short-term assay with S. typhimurium, used for detecting mutagens and carcinogens for higher

Tab. 3. Effects of various vegetable juices on the mutagenicity of Trp-P (10).

Conc. of Trp-P (μg/plate)	Vegetable juice	Mean No. of His^+ revertants/plate
200	None	382
0	None	25
200	Spinach	360
200	Celery	286
200	Cabbage	106
200	Lettuce	327
200	Radish	212
200	Turnip	126
200	Sprouts	357
200	Ginger	104

Test system : TA98 + S9 Mix

animals, including humans, it has become clearer that the process of metabolic activation is one of the important procedures that should be included in this assay system. In the process of metabolic detoxification in the living body, the liver plays an important role. In many cases, external toxic substances are expected to be metabolized into hydrophobic ones which are excreted to the outside from the body. During metabolic changes of chemical compounds, they are sometimes converted to mutagenic compounds. Some inhibitors can block these metabolic activations of promutagens and procarcinogens, thus suppressing mutagenesis and carcinogenesis.

The desmutagenic factor with NADPH-oxidase activity, purified from cabbage juice, also has an inhibitory effect on the metabolic activation of mutagens by the S-9 fraction from rat liver homogenate.

In general, it is very difficult to distinguish whether desmutagenic activity of a certain substance is due to inhibition of metabolic activation or to direct inactivation of mutagens. For mutagens acting without metabolic activation by S-9 mix, the possibility that their inhibition is due to blocking of metabolic activation can be excluded.

Inactivation of Activated Mutagens, Including Scavenging

When promutagens are activated by liver homogenates, they may become highly active mutagens. The effective screening of desmutagens may be possible by using activated mutagens.

Some desmutagens have a scavenging or binding activity for mutagens. Burdock juice has inhibitory effects on the mutagenicity of Trp-P-1, Trp-P-2, 2AA, and ethidium bromide. This desmutagenic activity of burdock juice is not affected by heating at 100°C for 15 min. The active principle of burdock juice was partially purified. The factor was absorbed to DEAE-cellulose but not to CM-cellulose, indicating that it may be a strong anionic polyelectrolyte. This suggests that the desmutagenic activity of burdock juice may be due to the ability of burdock fibers to adsorb mutagens.

Purified fibers were prepared from various vegetables (43). These fibers were added to water solutions of Trp-P-1, Trp-P-2, or Glu-P-1 at concentrations of 1-5% (w/w), and the mixtures were kept at room temperature for several hours. The mutagenic activities of pyrolysate

Tab. 4. Purification of the desmutagenic factor from cabbage leaves (6).

Purification	Volume (ml)	Activity	Protein (mg/ml)	Specific activity/mg
I. Dialyzed 9,000 x g sup.	3,900	7,100	7.86	0.24
II. DEAE-cellulose	5,000	10,600	4.16	0.51
III. CM-cellulose	1.4	2,300	33.6	49.5
IV. Sephacyl S200	8.3	1.980	2.24	106.3
V. DEAE-Sephadex A25	3.6	2,050	2.08	123.3
VI. Second CM-cellulose	3.6	1,522	3.6	87