微生物の生産する新しいポリペプチド抗麦異原物質に関する基礎的研究

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研 究 組 織

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図・本館

［研究発表］

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［研究成果］

1.Introduction

There are many kinds of mutagens found in our environment. Most of them are thought to be related to cancer, genotoxicity and aging. The elimination of such mutagens from our environment is desirable from the viewpoint of human health. From this view point, we believe that it is important to study on antimutagenic

factors present in our environment, which are divided into two main classes. One type of factors is called as "desmutagen", and the other type of factor is called as "bio-antimutagen". In general, desmutagens inactivate or destroy mutagens directly or indirectly out of cells. For example, 1,4-dinitro- 2-methyl pyrrole, which is the main mutagen formed by the reaction of sorbic acid and sodium nitrite, was reported to be inactivated by the treatment with vitamin C . Natural antioxidative substances are generally classified into the excellent desmutagens. On the other hand, the mecanism of bio-antimutagenesis is explained to suppress the process of mutagenesis itself in the cells. At the present stage, bio-antimutagens are thought to act as the factors to induce non-mutagenic repairs, inhibit of mutagenic repairs, and inactivate of SOS repairs, and so on.

In this study, I focussed my attention on bio-antimutagens. Recently, 293 strains of Actinomyces were screened to detect the factors which have bio-antimutagenic effects on UV- or MNNG-induced mutagenesis using a microbial mutation assay systems, especially, using E.coli MP-1 (Fig.1). I paid an attention, especially, to the culture of Z-24 strain of Streptomyces which showed strong bio-antimutagenic effects on UV-induced mutagenesis. From these background, I started my project whose purpose is to isolate and identify the new type of bioantimutagens present in the culture of Z-24 strain, and also to clarify of the bioantimutagenic mechanisms.

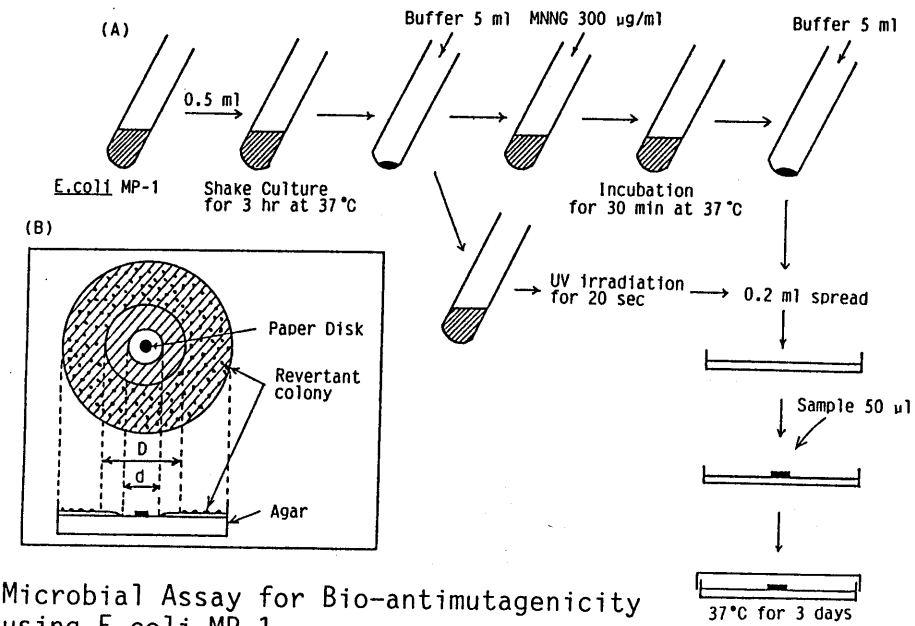

Fig.1 Microbial Assay for Bio-antimutagenicity using E.coli MP-1

2 Extraction and Isolation of Bio-antimutagen from the cultured medium of Streptomyces Z-24

At first, the cultured medium of 293 strains of Actinomyces have been screened for the evaluation of bio-antimutagenicity, and strong bio-antimutagenicity has been observed in the cultured medium of 8 strains. Fig.2 shows the scheme for screening, and Table 1 shows the bio-antimutagenicity of the metabolites of Streptomyces using E.coli MP-1 and B.subtilis NIG 1125. At the last step for screening, the liquid media of 9 strains, in particular, AM-1, AM-2, Z-24 and their mutants seem to contain strong bio-antimutagens.

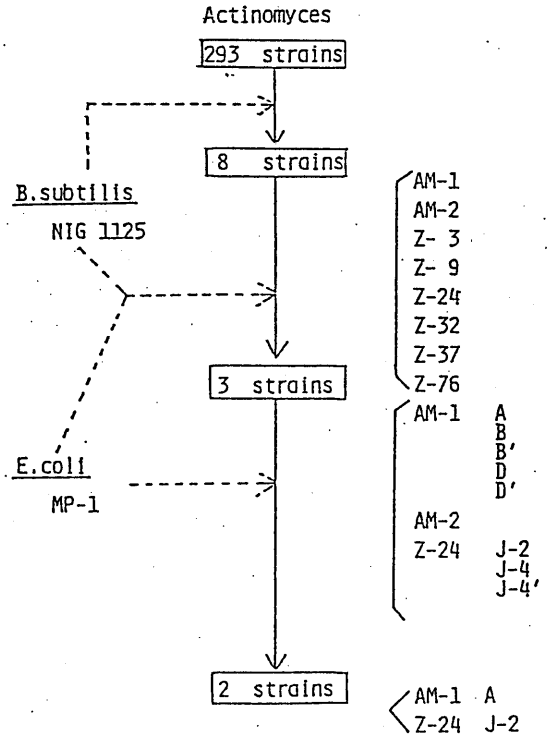

Fig.2 Scheme for Screening of Bio-antimutagenicity of Actinomyces

Mutagen	E. coli MP-1 $leu^- \rightarrow leu^+$				B. subtilis NIG 1125 $his^- \rightarrow his^+$	
	UV		NG			
	R	r	R	r	R	r
AM-1	34	–	31	–	10	–
AM-2	25	9	22	–	11	–
Z-3	42	32	50	38	30	12
Z-9	18	11	25	10	20	10
Z-24	40	18	41	20	41	11
Z-32	15	10	18	–	15	–
Z-37	19	14	–	–	14	–
Z-76	12	–	–	–	10	–
Co (500r)	38	10	32	15	20	12

Table 1 Bio-antimutagenicity of the Metabolites of Actinomyces

250 l cultured medium of Z-24 strain was centrifuged to remove bacterial cells and concentracted in vacuo to 1 l at Central Research Laboratories, Ajinomoto Co. ltd. Because the preliminary study indicated that antimutagens could be dissolved in MeOH, 15 g of the concentrated culture broth was extracted twice with 300 ml MeOH, filtered and concentrated under reduced pressure.

The extracts were separated into 4 fractions by a column chromatography over Amberlite XAD-2 with H_2O-MeOH as the solvent system. Each fraction was evaporated and assayed in E. coli MP-1 with UV. As summarized in Fig.3, antimutagenic fractions were separated using different type of column chromatography, and finally, the pure antimutagenic compound (Z-24) has been isolated by preparative HPLC using ODS-5 with 0.1 % HFBA-MeOH (6:4 v/v)(Fig.4). The yield of isolated substance was 20 μg by the determination with Tonnein-TR.

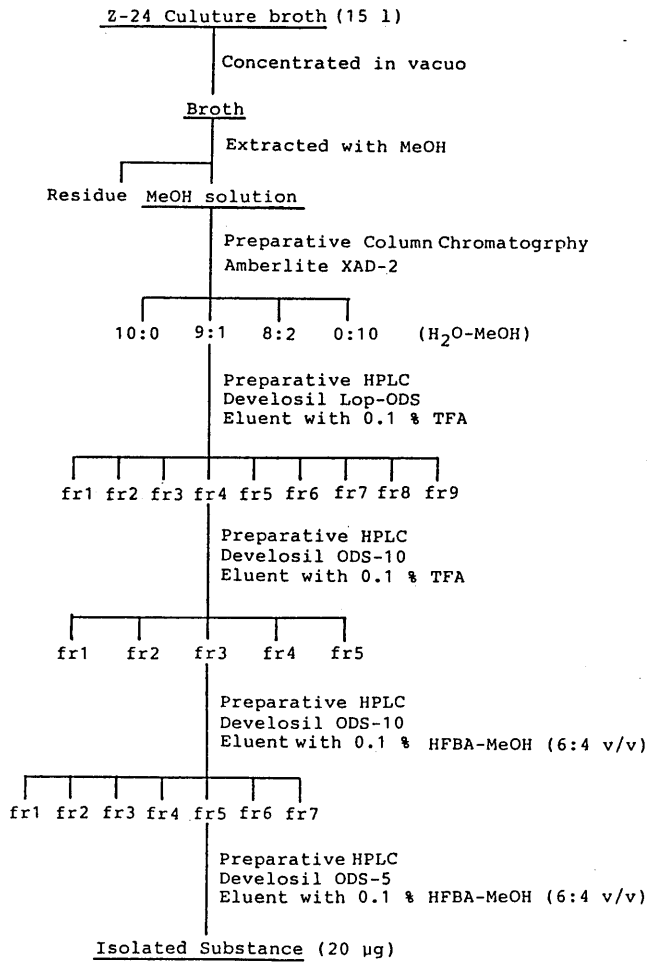

Fig.3 Purification and Isolation of Bio-antimutagens

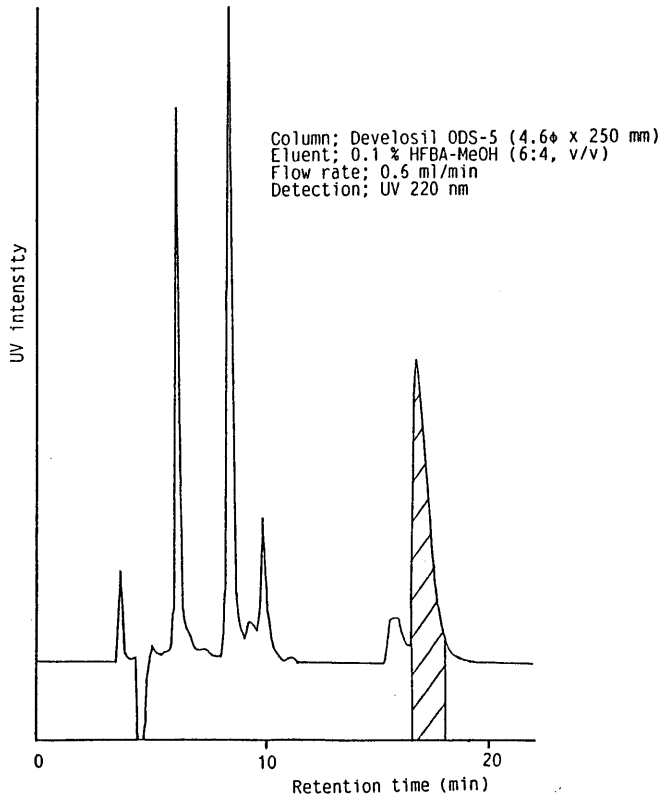

Fig.4 Preparative HPLC

3 Chemical Analyses of Z-24

The result of amino acid analysis indicated that active substance contains 13 kinds of amino acids and the composition is as follows;

Asp (1), Thr (1), Ser (1), Glu (2), Pro (8), Gly (3), Ara (1), Cys (2), Val (2), Leu (4), Ile (4), Phe (1), His (1).

Moreover **Z-24** was positive on ninhydrin reaction. These results suggest that **Z-24** seems to be a peptide type compound.

Next, I decided to try electrophoresis for determination of the molecular weight of **Z-24**, and amino acid sequencer in order to confirm the sequence of amino acid constituents of **Z-24**.

Molecular weight of **Z-24** was determined by SDS-PAGE. The

used gel was gradient gel ranging in concentration from 8 % to 25 %. From the result of electrophoresis, the molecular weight of Z-24 seemes to be less than 14,400 Da, and about 7,000 - 10,000 Da, by comparison with the molecular weight of the standard peptides such as Myoglobin, Myoglobin I, Myoglobin II, Myoglobin I & II, and Myoglobin III (Fig.5).

I tried to determine the NH_2-terminal amino acid sequence of Z-24 by Edman degradation with the use of an Applied Biosystem model 470 A gas phase sequencer. The PTH derivatives of the amino acids obtained at each cycle of the Edman degradation were tried for determination by HPLC, however, no PTH-amino acids from cycles 1 to 9 were detected. This results indicated that NH_2-terminal of Z-24 may be blocked or be different from α-amino acid.

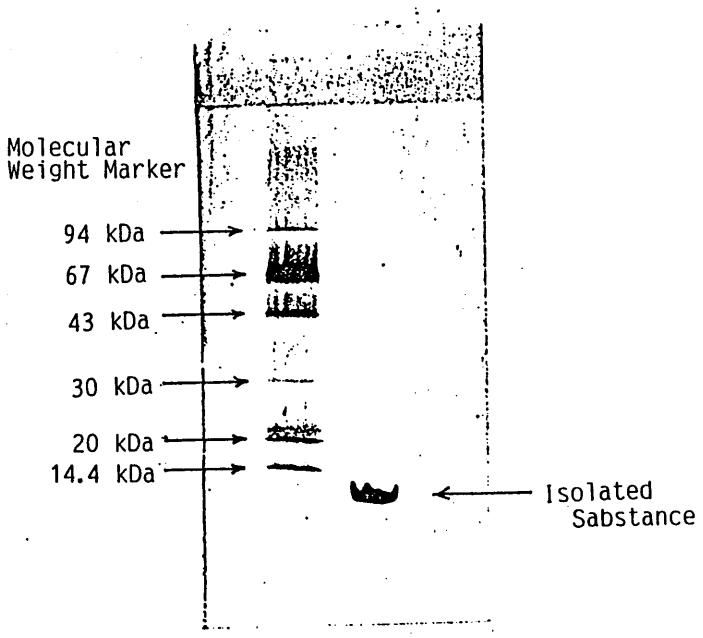

Fig.5 SDS-PAGE

4 Bio-antimutagenicity of the isolated substance

During isolation and purification processes, spot tests were employed as the bioassay system to evaluate bioantimutagenicity of each fraction. This test is very simple and detectable for bioantimutagenic activity, however, it's very difficult to evaluate the bio-antimutagenicity quantitativly. For the purpose of quantification of bio-antimutagenicity, bacterial cells were spread onto the base agar and revertant colonies were counted, in order to evaluate activity of Z-24.

Table 2 shows the results of bio-antimutagenic activity of Z-24 by spot test, and Fig.6 shows by the quantative bio-antimutagenicity obtained by counting the number of revertant colonies (soft-agar method). Cobalt Chrolide, which is known as the strong bio-antimutagen, was used as the standard for comparation with Z-24. In the case of the spot test, only 0.5 g of Z-24 was applied on the gel plate, however the bio-antimutagenic activity is similar to that of Cobalt Chrolide. And the result obtained by soft-agar method also indicates that the isolated substance have strong bio-antimutagenic activity but have little effects on viability of the cells at low concentration.

Table 2

	Sample Weight used for Assay (μg)	D (cm)	d (cm)
Isolated sabstance	0.5	34	17
$CoCl_2 \cdot 6H_2O$	500	41	19
H_2O		10	9

Bio-antimutagenic Activity on Spot Test using E.coli MP-1 Induced by UV-irradiation

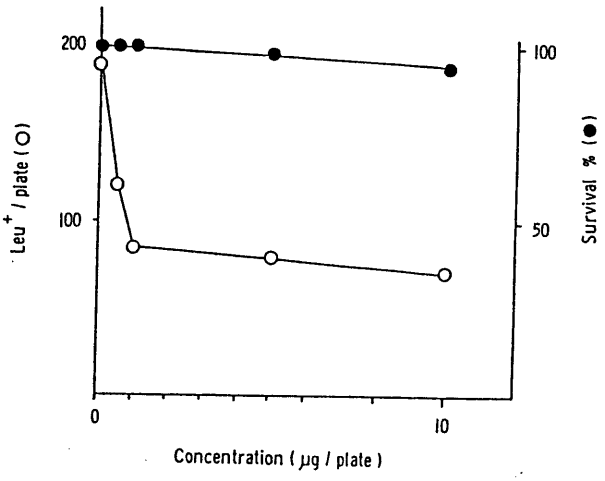

Fig.6 Bio-antimutagenicity of Z-24 on E.coli MP-1 induced by UV-irradiation

5 General Discussion

As our knowledge of the interlocking roles of mutational changes in genes and the development of cancers has increased, it has become increasingly clear that bio-antimutagenesis and desmutagenesis could have play an important role in lowering cancer incidence. Therefore I paid an attention on bioantimutagens, especially, on bioantimutagenic substances isolated from the cultured medium of Z-24 strain of Streptomyces which showed strong bioantimutagenic effects on UV-induced mutagenesis.

In the present study, investigation on bio-antimutagen present in the cultured medium of Z-24 strain has been carried out, and finally, the 20 g of bio-antimutagen has been isolated, and chemical and physical properties of the isolated substance, Z-24, has also been characterized by instrumental analyses. In order to determine the chemical structure of Z-24, SDS-PAGE and Gas Phase sequencer were used. Using SDS-PAGE, the molecular weight of Z-24 was determined to be less than 14,400 Da, probably, it is about 7,000 - 10,000 Da. On the other hand, Gas Phase Sequencer was used in order to determine the NH_2-terminal amino acid sequence of Z-24. The PTH derivatives of amino acids obtained each cycle of the Edman degradation were tried for determination by HPLC, however, no PTH-amino acids from cycles 1 to 9 was detected. From this result, it is thought that the terminal amino acid residue might be blocked or be not an α-amino acid.

Bio-antimutagenic activity of Z-24 was determined by spot test and soft-agar method, and Z-24 showed remarkable bioantimutagenic activity even at low concentration. However bio-antimutagenicity of Z-24 has not yet fully interpreted by these two bioassay systems, since decrease of number of Leu colony can be resulted from specific growth inhibition of the leu bacteria by agent. Therefore, it is necessary to confirm that the reduction of induced Leu colonies is due to the effects of bio-antimutagenicity in the course of radiation-induced mutagenesis and not due to simple growth inhibtion of the Leu bacteria by Z-24. In order to clarify this question, reconstruction experiments shoud be carried out.

The antimutagenic activities of Z-24 have been investigated using E.coli MP-1 induced by MNNG and UV irradiation at the present stage, however, bio-antimutagenicity of Z-24 shoud be investigated using other microbial mutagenicity assay systems and also other in vitro system to confirm the mechanisms of bio-antimutagenicity of Z-24. Because there are strong correlation between bio-antimutagenesis and anticarcinogenesis, Z-24 can be used as the anticancer drug, however, further detailed examination shoud be carried out.

Abbreviation

Da : dalton
DMSO : dimethyl sulfoxide
Fig. : figure
Fr. : fraction
HFBA : hepta fluorobutyric Acid
HPLC : high performance liquid chromatography
HSA : human serum albumin
M3 : antibiotic medium 3
MeOH : methanol
MNNG : N-methyl-N'-nitro-N-nitrosoguanidine
OD : optical density
PAGE : polyacryl amido gel electrophoresis
PTH : phenylthiohydantoin
SOD : sodium dodecyl sulfate
TFA : trifluorobutyric acid
UV : ultra violet
Z-24 : the bioantimutagen which was isolated from the cultured medium of Z-24 strain of Streptomyces