KOREAN J. FOOD & NUTRITION
Vol. 12, No. 4(1983)

DNA-Breaking Action of Some Biologically Active and Other Nitrogen Compounds

Jin Ha Lee and Seung Si Ham

Department of Food Science and Technology, College of Agriculture, Kangweon National University
(*Received August* 24, 1983)

數種의 生理活性物質 및 含窒素化合物의 DNA 切斷作用

李 鎭 夏 · 咸 昇 市

江原道 春川市 江原大學校 農科大學 食品工學科
(1983년 8월 24일 수리)

國文 抄錄

數種의 核酸構成成分, 아미노酸, 尿素誘導體와 같은 生理活性物質 및 含窒素化合物의 DNA에 대한 作用과 그 作用에 無機ion의 영향을 檢討하였다. PTU, Cys-SH는 金屬 ion없이도 DNA 切斷能力을 가지고 있었고, Tyr, Phe, Trp는 $CuSO_4$存在 下에서 약한 DNA damage 誘起作用이 있었으며 5mM 이상의 Cys-SH와 500μM $CuSO_4$의 混合液은 強한 DNA 切斷作用을 나타냈다. Sn^{2+}를 제외한 500μM의 無機鹽은 DNA에 대한 절단능력이 없었으나, Cu^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, Sn^{2+} 등은 數種의 아미노酸의 DNA breaking action에 영향을 미치고 있음이 確認되었다.

Introduction

Living organisms contain basic nutrient compounds, biologically active and other essential substances. Most foods are obtained from many kinds of living bodies. Constituents of the living bodies generally take part in their metabolisms or carry out a biologically action which may be mutagenic or carcinogenic and anticarcinogenic activities. In particular, the investigation on the DNA breaking action by the biologically active compounds is note worthy since the breakage of double strand DNA molecules is closely related to the important events to the living organisms. Omura *et al*, reported that the decreased viscosity of DNA solution was caused by ascorbic acid and its derivatives[1] and amino and thiol reductones[2] in the presence of Cu^{2+}. The breakage of DNA by aromatic reductones was also tested *in vtiro*.[3]

Some food contituents and products during food processing or storage possess mutagenic activities. Trp-p-1, Trp-p-2, Glu-p-1 Glu-p-2 and 1-amino-4-phenylpyridine formed by pyrolysis of tryptophan(Trp), glutamic acid (Glu) and phenylalanine (Phe) are strong carcinogenic compounds[4-6]. Lee[7,8] ascertained that the extracts of apricot and peach kernels broke the double strand of calf thymus DNA and that guanine analogs formed in the reaction of triose reductone with guanine also possessed breaking action in the presence of cupric ion.

In the present paper, we examined the effect of nucleic acid constituents, ureas, amino acids and metal ions on the breakage of calf thymus DNA.

Materials and Methods

Chemicals : Nucleic acid related compounds and amino acids were purchased from Khojin and Wako Chemical Co. Ltd., respectively and other reagents employed in this study were the best quality.

Calf thymus DNA: DNA was purchased from Sigma Co. and was prepared according to the SDS method.[9] DNA(500 $\mu g/ml$)was dissolved in 25 mM NaH_2PO_4-12.5 mM Na_2HPO_4 buffer solution (pH 6.6) and applied to the breaking test.

Reaction of the DNA breakage: Each sample of nucleic acid constituents, amino acids and ureas was mixed with the double strand DNA solution and incubated at 37°C for 30 to 240 min. The reaction mixtures were examined their abilities to break the DNA on agarose slab gel electrophoresis. The samples were dissolved in dimethylsulfoxide(DMSO) because of their insolubilities in distilled water. The reaction and the electrophoresis were carried out by Lee's method[8].

Effect of metal ions on DNA breaking action: The effect of metal ions and the combination effect of metal ions with the nucleic acid constituents, amino acids and ureas on the breakage of DNA were determined. The metal ions employed in this study were $Cu^{2+}(CuSO_4)$, $Fe^{2+}(FeSO_4)$, $Fe^{2+}[Fe_2(SO_4)_3]$, $Mg^{2+}(MgSO_4)$, $Pb^{2+}(Pb(OAc)_2)$, $Al^{3+}(AlCl_3)$, $Hg^{2+}(HgCl_2)$, $Mn^{2+}(MnSO_4)$, $Ni^{2+}[Ni(NO_3)_2]$, $Zn^{2+}[Zn(NO_3)_2]$, $Sn^{2+}(SnCl_2)$ and $Ca^{2+}(CaSO_4)$.

Results and Discussion

DNA breaking action of ureas : The reaction mixture of calf thymus DNA with 30 mM ureas was incubated at 37°C for 4 hr and applied on agarose slab gel. Fig. 1 indicates the electrophoregrams of the reaction mixtures of DNA with phenylurea(PU, Ⅰ) and DNA with methylurea(MU, Ⅱ). The reaction mixtures reacted for 30 to 240 min did not show the double strand breakage of the DNA. The reaction mixture of DNA and ethylurea(EU) was shown a similar trend to the one of MU. DNA-breaking actions of thiourea (TU) and phenylthiourea (PTU) show in Fig. 2. TU did not show a breaking ability of DNA,

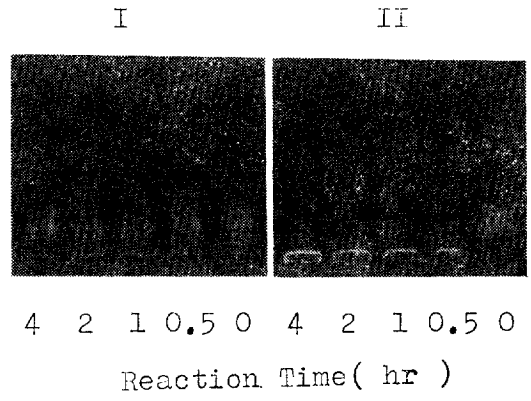

Fig. 1. Agarose gel electrophoregrams of the double strand breaks on calf thymus DNA treated with 30 mM PU(Ⅰ) and MU(Ⅱ) at 37°C for 240 min.

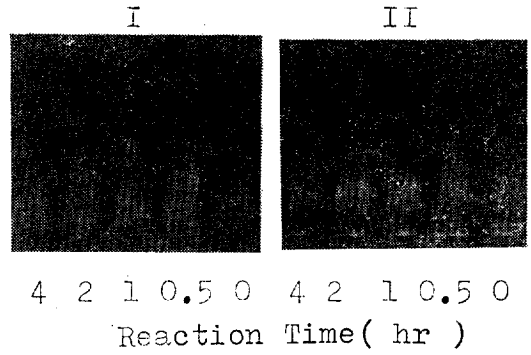

Fig. 2. Electrophoregrams of the DNA treated with 30mM TU(Ⅰ) and PTU(Ⅱ) at 37°C for 240 min.

however, PTU possessed a weak DNA-breaking activity. These results indicate that the thio and phenyl groups of ureas do not seem to have a strong double strand breakage action on the DNA. Most substituted ureas are shown nontoxic by acute oral administration while TU is known a carcinogen[10]. The main target organs of TU and similar thioamides are the thyroid and in some instances the liver. The action of TU on the thyroid is thought to be due to an in-

terference with the synthesis of thyroxine leading to an imbalance of the pituitary-thyroid grand relationships. Thus the increased flow of thyrotropic hormones is generated by the pituitary, stimulating thyroid growth and contributing to tumor formation.

DNA-breaking action of nucleic acid constituents: Fig. 3 shows the electrophoretic patterns of the reaction mixtures of DNA and 30mM nucleic acid constituents, adenine(Ade), adenosine(Ado)and guanosine(Guo) at 37°C for 30 to 240 min. These nucleic acid compositions did not show a DNA-breaking ability. Guanine, GMP, CMP and AMP could not examined since they did not dissolved in DMSO at 50°C. Guanine analogs modified by triose reductone have a strong breakability on double strand DNA in the presence of cupric ion[8] and various nucleoside and nucleotide analogs were attempted to utilize as anticarcinogenic agents by applying the mechanism of their biological activities on many bacterial tests.[11]

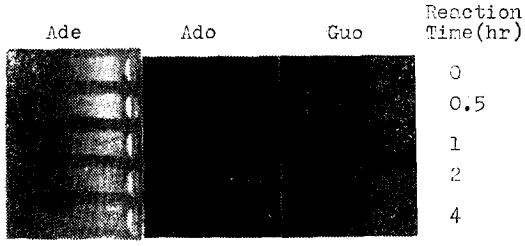

Fig. 3. Electrophoregrams of the DNA treated with 30 mM Ade, Ado and Guo at 37°C for 240 min.

DNA-breaking action of amino acids: The DNA-breaking action of amino acids such as glycine, alanine, valine, leucine(Leu), iso-leucine, serine, threonine, aspartic acid, glutamic acid, lysine, arginine, phenylalanine, proline, histidine, methionine (Met), tryptophan(Trp), tyrosine(Tyr) and cystine(Cys-SH) were examined. The double strand breakage of the illustrated amino acids except Cys-SH on DNA were not observed on a gel electrophoresis. Fig. 4 indicates the DNA-breaking action of the various concentrations of Cys-SH. Among these concentrations, 30mM Cys-SH only has a strong ability to break the DNA. It is postulated that the double strand breaks of the DNA by Cys-SH are to be attributed to the strong reducing power of its sulfide group. From this result, it may be concluded that most amino acids do not cause a mutagenicity on bacteria and other organisms. However, the pyrolysates of Trp, Phe and Glu possess a strong carcinogenic and mutagenic activities.[4~6] In additions, a pyrolytic product of protein, amino-α-carbolines, induce a mutagenic action on *Salmonella typhimurium* system.[12]

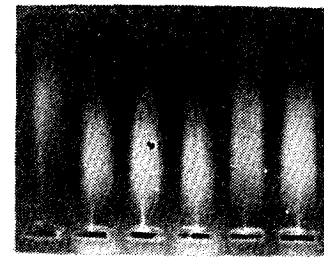

Fig. 4. Electrophoregram of the DNA treated with various concentrations of Cys-SH at 37°C for 120 min.

Effect of metal ions on DNA-breaking action: The DNA-breaking action of metal ions such as Cu^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+}, Pb^{2+}, Al^{3+}, Hg^{2+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Sn^{2+} and Ca^{2+} were examined at 37°C. The reaction mixtures of 500 μM metal ions and calf thymus DNA were incubated at 37°C for 2hr and the reacted mixtures were developed on agarose slab gel. Fig. 5 indicates the DNA strand breaks by 500μM of metal ions(Ⅰ) and 30mM Met(Ⅱ) and 30mM Leu (Ⅲ) in the presence of 500μM the above-stated metal solutions. All but Sn^{2+} solution did not possess the ability to give rise to the DNA lesions. Among the mixtures, Cu^{2+}, Mn^{2+}, Ni^{2+} and Sn^{2+} accelerated the DNA damage by the combination action 30mM Met and 30mM Leu. Lee[7,8] reported that 1,000μM $CuSO_4$ did not in-

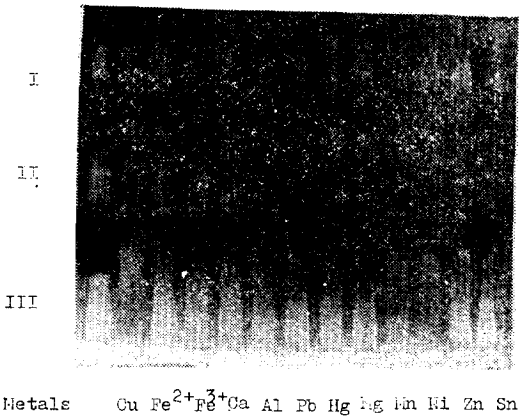

Fig. 5. Electrophoregrams of the DNA reacted with 500 μM metal ions(Ⅰ) and 30 mM Met(Ⅱ) and 30 mM Leu (Ⅲ) in the Presence of 500 μM Metal Ion Solutions at 37°C for 120min.

duce a double strand scission on DNA and that the cupric ion accelerated the DNA damage by guanine analogs and the extracts of apricot and peach kernels. Consequently, they concluded that the concentration of the inorganic salts which might affect to the DNA-breaking action by organic compounds could be employed so far as 1,000μM. The double strand breaks of DNA by Cys-SH and other some amino acids in the existence of 500μM $CuSO_4$ at 37°C for 120 min are shown in Fig. 6. Cys-SH, at the concentrations of 5mM and over of it, caused the cleavage of the DNA in the presence of 500μM $CuSO_4$, and 1mM Cys-SH also attested a weak DNA breakability. 15mM Tyr, Phe and Trp which are aromatic amino acids in the presence of 500μM $CuSO_4$ possessed the activity to cause the DNA lesions. The double strand breaks of DNA by the mixtures of other compounds in the presence of the inorganic salts were not observed. From these results, it is suggested that the DNA strand breaks by some amino acids are affected by the presence of Cu^{2+}, Mn^{2+}, Ni^{2+} and Sn^{2+}.

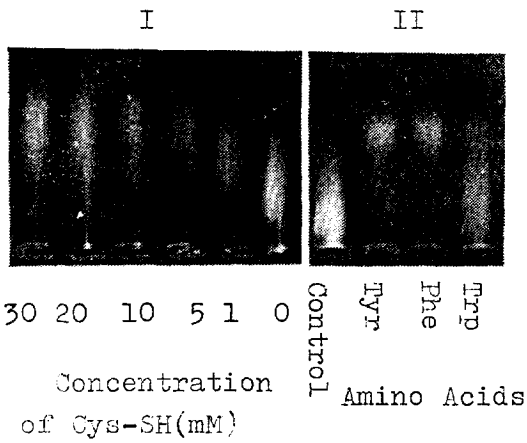

Fig. 6. Elcctrophoregrams of the DNA reacted with various concentrations of Cys-SH in the presence of 500 μM $CuSO_4$(Ⅰ) and 15 mM Tyr, Phe and Trp in the presence of 500 μM $CuSO_4$(Ⅱ).

The metal having the greatest potential for causing disease and being released to the environment may be bioconcentrated and thus enter the food chain and they are those which accumulate in the body. Metal and metal ions are known to affect diversely in biological actions; heavy metals cause a cancer and some other metals accelerate a mutagenic action.

In the reactions of nucleic acid constituents with some inorganic salts, the complexes of IMP-Pb, IMP-Cd, IMP-Co, IMP-Zn, IMP-Ni, IMP-Mn,[13,14] ATP-Mn, ATP-Zn, ATP-Ca, ATP-Mg,[15] Cu-ITP, Cu-GTP, Cu-CTP, Cu-UTP, Cu-TTP,[16,17] Cu-Gua and Cu-Cyt[18] were isolated and their crystal structures, stabilities and their binding sites with metal ions were investigated by X-ray diffractometer and other methods. These studies on the complexes of nucleic acid related compounds and metal ions were mostly performed to confirm the reaction mechanisms and their structures. The effect of the numerous inorganic and organic compounds on the physiological functions such as nucleic acid and protein synthesis were studied, however, there was few investigations on the direct reaction of macromolecular DNA with inorganic compounds.

Summary

The effect of the nucleic acid related compounds, amino acids and ureas on the breakage of calf thymus DNA were investigated with or without inorganic salts. PTU and Cys-SH possessed the ability of DNA strand breaks without metal ions. Tyr, Phe and Trp induced a weak DNA lesions in the presence of $CuSO_4$. Cys-SH with concentrations of 5mM in the presence of metal ion, $CuSO_4$, showed the strong ability to break the DNA. Various metal solutions(500μM) except Sn^{2+} did not show the DNA-breaking action. The DNA strands were damaged by some amino acids in the presence of Cu^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+} and Sn^{2+}.

Acknowledgment

This research was supported by the 1982 research grant from Kangweon National University.

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