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Studies on DNA Mutagenesis Mediated by Maillard Reaction Products

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ABSTRACT

A glucose-lysine Maillard model system was prepared and its reaction products (MRPs) obtained. Reaction of the 0.025M to 1M glucose-lysine MRPs with plasmid pUC12, resulted in lowered transformation efficiency of pUC12 throughout the reaction time which ranged from five minutes to twenty-four hours. Attempts to compare the pUC12 DNA pattern after being incubated with the MRPs, showed no size change of the DNA as evidenced by gel electrophoresis. Following increased incubation time of MRPs and pUC12 DNA from four to twelve days, showed transformation efficiencies of the pUC12 to be 3.2% and 0.15%, respectively. Transformation efficiencies of pUC12 incubated with MRPs for 12 days were several folds lower than that incubated for four days. Following denaturation of the incubation mixtures of pUC12 and MRPs by ethanol precipitation and renaturation in Tris-EDTA buffer, the transformation efficiency of pUC12 was increased by more than ten folds. This phenomenon was also found in those of the pUC12 incubated with glucose-lysine MRPs within twenty-four hours.

Key words: Maillard reaction, DNA mutagenesis, transformation efficiency.

INTRODUCTION

The nonenzymatic reaction of reducing sugars with the amino groups of proteins was first described by Maillard in 1912[1]. The endproducts are characteristically fluorescent, yellow-brown in color, and are able to crosslink proteins inter- and intra-molecularly. Maillard reaction products(MRPs) were first shown to occur during the aging of stored food but recently have been found in long-lived human proteins such as collagen[2] and the lens crystallins[3]. Studies of diabetic patients, have shown an accelerated rate of nonenzymatic browning that may contribute to the earlier onset of cataracts and atherosclerosis[4]. A role in DNA mutations were found in diabetes-associated teratogenesis in transgenic embryos, twofold increase in the mutant frequency of the *lacI* transgene in fetuses that developed in a mild diabetic environment compared under normoglycemic conditions[5].

Recent investigations have demonstrated that

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amino groups of nucleic acids can serve as substrates for modification by reducing sugars, leading to spectral changes similar to those described for the nonenzymatic browning of proteins[6]. The incubation *in vitro* of f1 phage DNA with either glucose or glucose 6-phosphate (G-6-P) resulted in a time and sugar concentration loss in transfection capacity[6]. Incubations of G-6-P with plasmid DNA, pBR322, decreased the transformation efficiency of the DNA and results in plasmid mutations, comprising of DNA insertions and deletions[7]. Exposure of target plasmids to elevated G-6-P levels results in an increase in plasmid mutations which have been associated with the amount of G-6-P accumulation[8]. Increase of $\gamma\ \delta$ transposition has also been associated with the amount of G-6-P accumulated in host cells[9]. A model reaction of G-6-P with the amino groups of lysine to form reactive intermediates which are capable of forming covalent adducts with single- or double- stranded DNA to form acid-stable complexes[10].Furthermore, advanced glycosylation endproducts react with DNA *in vitro* and transfect into murine lymphoid cells leading to transposition of an Alu-containing element[11].

However,the detailed aberration of the DNA after the reaction with Maillard reaction intermediates are not yet clearly understood. Therefore, the purpose of this study was to use model reactions of glucose and lysine, or proteins to form different MRPs, then to examine the subsequent effects on the plasmid pUC12 DNA.

MATERIALS AND METHODS

I. *Preparation of Glucose-Lysine Maillard Reaction Products (MRPs)*

Glucose and lysine at concentrations of: 0.0125M, 0.025M, 0.05M, 0.1M, 0.2M, 0.4M, 0.6M and 1.0M were dissolved in 0.1M potassium phosphate buffer (pH 7.0), respectively. All solutions were sterilized by filtration through a millipore filter prior to use. Glucose-lysine mixtures were incubated at 37℃, 200rpm, and kept for two months. They were subsequently aliquoted and stored at -20℃ until further analysis.

II. *Incubation of Proteins with Glucose*

Ten mg/ml of BSA(bovine serum albumin, Sigma B-2518), lens crystallin protein (Sigma C-4163) and lysozyme (Sigma P-6876) were dissolved in 0.5ml of 0.2M potassium phosphate buffer (pH7.0), respectively. Each type of protein was mixed with 0.5ml of 0.2M D-glucose in 0.2M potassium buffer, pH7.0. The protein-glucose mixtures were incubated at 37℃, 150rpm, and kept for two months. The reaction products were aliquoted and stored at -20℃ for further use.

III. *Microorganisms, Plasmid and Medium*

Host cell for transformation was *Escherichia coli* DH5α , whose genotype is F- *end*A1 *hsd*R17(*r*k- ,*m*K-) *sup*E44, *thi*-1 λ *rec*A1 *gyr*A *rel*A1 Δ (*argF-lacZYA*)- ∪ 169 φ 80d*lacZ* ΔM15. The plasmid in use is pUC12, whose molecular size is 2.7 kb and whose selective genetic markers are Ap^r, $lacZ^+$. The medium for transformation and plasmid preparation was 2YT(1.6% Bacto tryptone, 1% yeast extract and 0.5% NaCl).

IV. *Reaction of the Plasmid pUC12 DNA with the MRPs*

One microliter of varied concentrations of MRPs were mixed separately with 1μl pUC12 DNA (1μg/μl). Each mixture was incubated at 37 ℃,was then directly added to the competent cells and subjected to plasmid transformation. Incubation of pUC12 DNA without MRPs was used as positive controls, the transformation efficiency of which was 100%.

V. *Plasmid Purification and Transformation*

Plasmid pUC12 DNA was isolated according to the method of Birnboim[12]. *E. coli* DH5 α transformation was carried out according to the

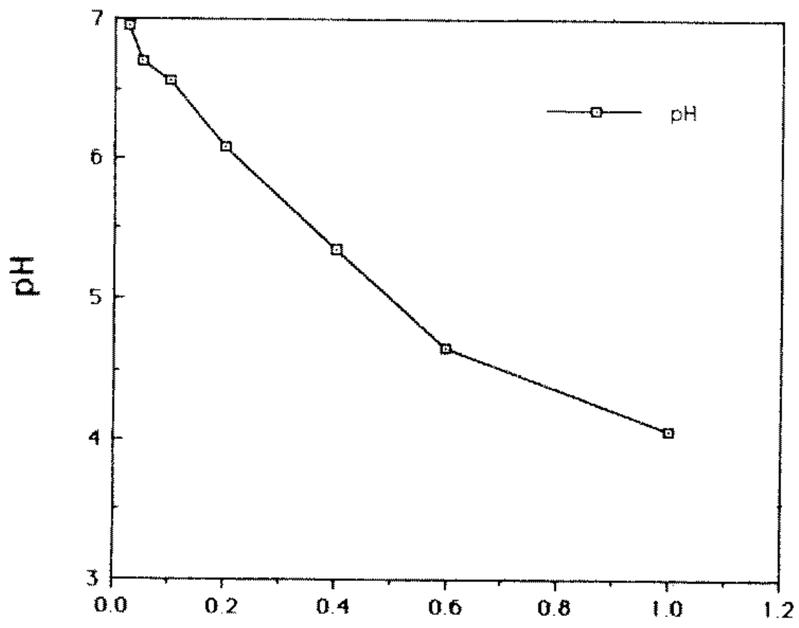

Figure 1. The end pH of the glucose-lysine MRPs at different reactant concentrations incubated at 37 ℃ for two months.

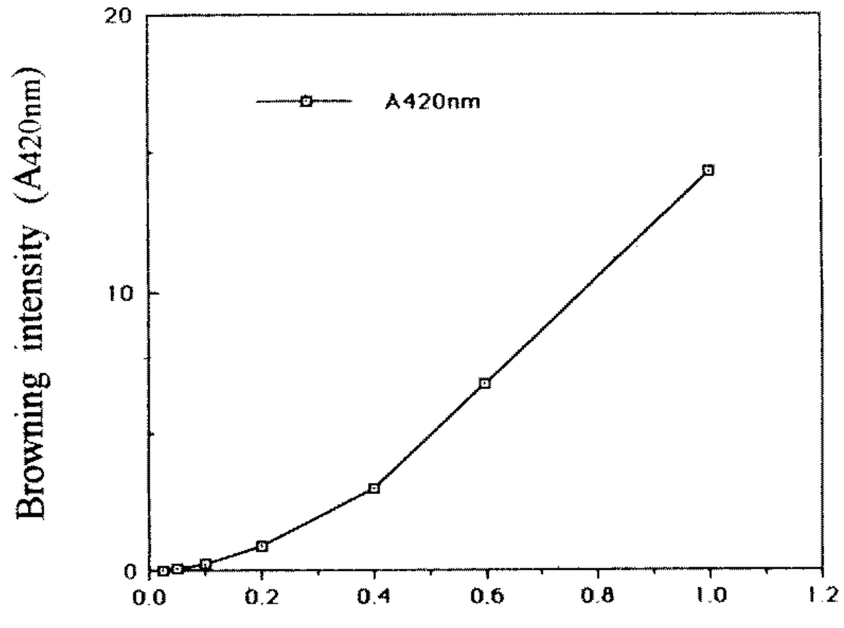

Figure 2. Browning intensity of the glucose-lysine MRPs at different reactant concentrations incubated

method of Maniatis[13] with minor modifications. The overnight *E. coli* culture was transferred to 100ml 2YT and incubated at 37℃, 200 rpm. Harvest of the cells was at A_{550} of 0.2-0.5(about 10^7-10^8 cells/ml). The cells were centrifuged (5000rpm) for 20min at 4℃, and resuspended in 10ml of precooled 100mM $CaCl_2$, 10mM Tris, (pH8.0). They were placed in an ice bath for 30min. The cells were harvested and resuspended in 2ml precooled $CaCl_2$ (100mM), 10mM Tris, (pH8.0) as competent cells.

DNA was added in 100μl of competent cell suspension and maintained at 0℃ for 30 min with occasional gentle agitation. Following 2 min of heat shock at 42℃, 0.1ml 2YT was added at 37 ℃ and the cells incubated for 1 hour. The transformed cells were subsequently plated in 2YT selective medium containing 100 μg/ml ampicillin, 0.2mM IPTG(isopropyl-1-thio-β-galactoside), and 0.004% Xgal(5-bromo-4-chloro-3-indolyl-β-galactoside).

VI. *Determination of Fluorescence of MRPs*

Fluorescence emissions were measured on a Jasco spectrophotometer (uviDEC-34). Incubation mixtures showing absorbance changes were diluted with distilled water. The recordings were determined at an emission wavelength of 420nm.

RESULTS

I. *Characteristics of the MRPs*

The pH and the browning intensity of the glucose-lysine MRPs reacted at different equimolar concentrations are shown in Figure 1 and Figure 2, respectively. The pH decreased and browning intensity increased with reaction time and concentrations of sugar and lysine. The end pH reached pH 4.06 and browning intensity A_{420} was 14.29 in the 1.0M glucose-lysine MRPs. These MRPs were chosen to be plasmid reactants. MRPs of glucose in combination with BSA, lens crystallin protein and lysozyme, did not exhibit measurable differences related to pH and browning intensity.

II. *Twenty-four Hour Reaction of the Plasmid pUC12 DNA with the Glucose- Lysine MRPs*

Different concentrations of MRPs were mixed separately with pUC12 DNA. Each mixture was incubated at 37℃ for 5min, 30min and 24 hours before subjecting to plasmid transformation. The transformation efficiencies of the pUC12 DNA incubated with the MRPs ranged

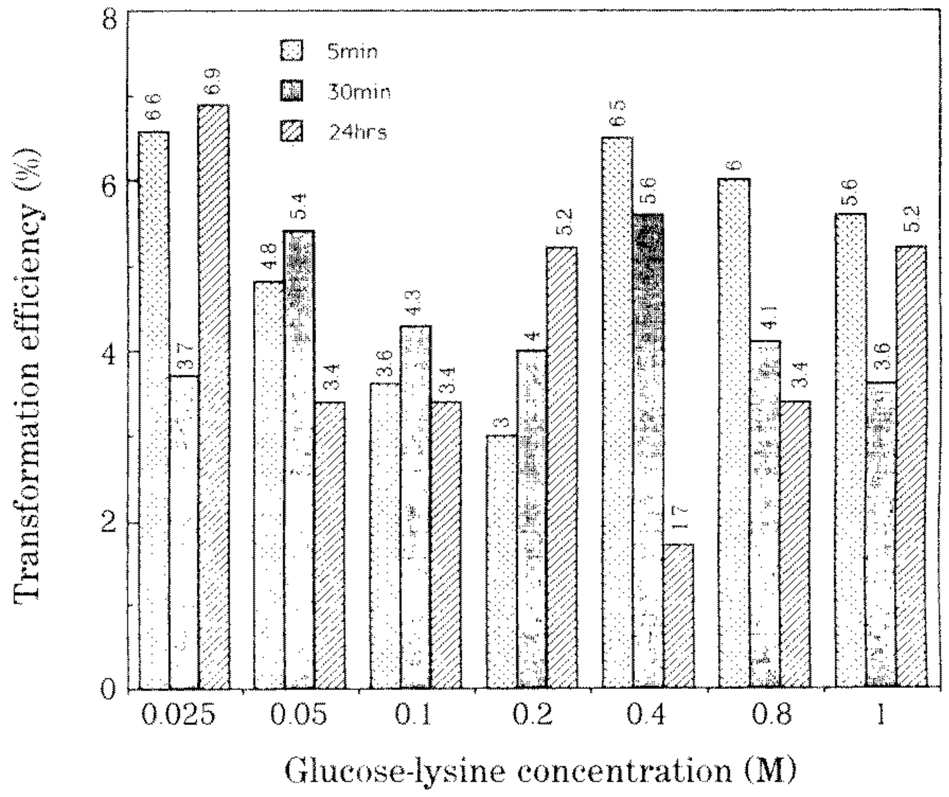

Figure 3. Transformation efficiencies of the pUC12 DNA incubated with the different concentrations of glucose-lysine MRPs for 5min, 30min and 24 hours. Incubation mixtures were subjected to direct transformation.

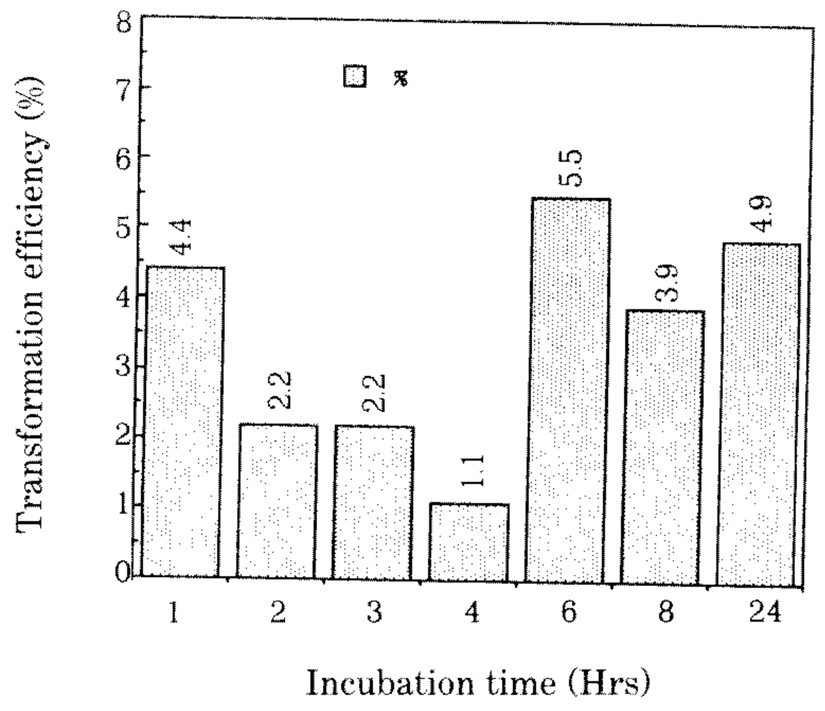

Figure 4. Transformation efficiencies of the pUC12 DNA incubated with 1M glucose-lysine MRPs for 1, 2, 3, 4, 6, 8 and 24 hours. Incubation mixtures were subjected to direct transformation.

between 1% and 7% (Figure 3). Transformation efficiency was not increased either as an integral function of the concentrations of the MRPs or the length of the incubation time. The 0.025M glucose-lysine MRPs reacted with pUC12 for 5 minutes expressed only 6.6% of transformation efficiency. The 1.0M glucose-lysine MRPs resulted in 5.6% of transformation efficiency.

Figure 4 shows the transformation efficiencies of pUC12 following reaction with 1.0M glucose-lysine MRPs for one, two, three, four, six, eight and twenty-four hours being 4.4%, 2.2%, 2.2%, 1.1%, 5.5%, 3.9% and 4.9%, respectively. The reaction mixtures of pUC12 and 1.0M glucose-lysine MRPs of the different reaction time were also examined by agarose gel electrophoresis, as represented at lanes 3 through 9 in Figure 5. No apparent size difference was found. Lane 2 and lane 10 are pUC12 positive controls; lane 1 and lane 11 represent the λ-*Hind*III digested fragments.

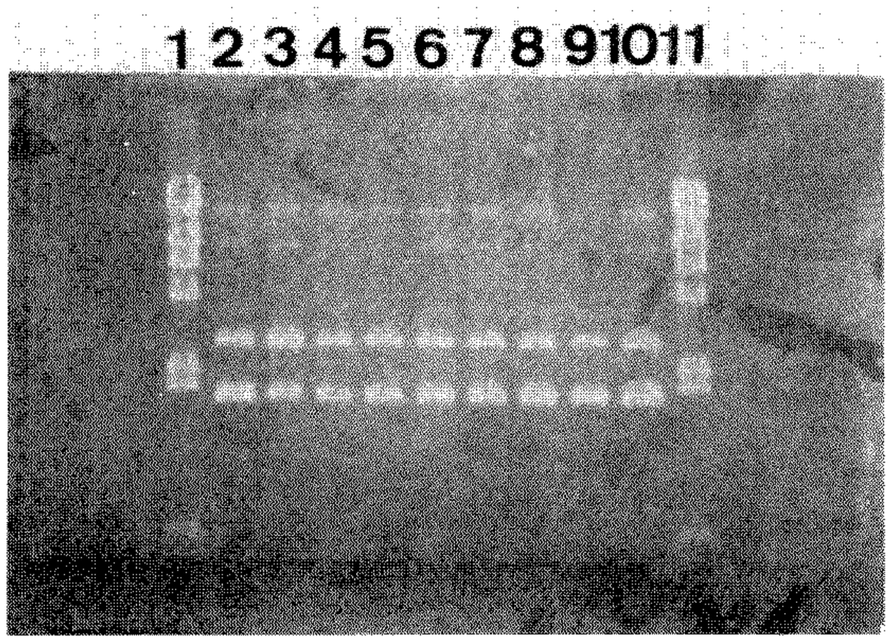

Figure 5. Agarose gel electrophoresis of the incubation mixtures of pUC12 DNA and 1M glucose-lysine MRPs as in Fig 4. Lane 1 and lane 11 were λ-*Hind*III fragments; lane 2 and lane 10 were pUC12 DNA positive controls; lane 3 to lane 9 were pUC12 incubated with 1M glucose-lysine MRPs for 1, 2, 3, 4, 6, 8 and 24 hours, respectively.

III. *The Transformation Efficiency as Determined after Precipitation of the Incubation Mixtures of pUC12 and Glucose-Lysine MRPs*

The incubation mixtures of pUC12 and different concentrations of MRPs at 6, 12 and 24 hours of incubation time were precipitated in 70% ethanol at -70℃ for two hours. pUC12 was subsequently centrifuged and resuspended in 1mM TE (Tris-EDTA, pH 8.0). Resuspended pUC12 DNA was added to the competent cells and subjected for transformation treatment. Transformation efficiencies are given in Figure 6. The transformation efficiencies for pUC12 react-

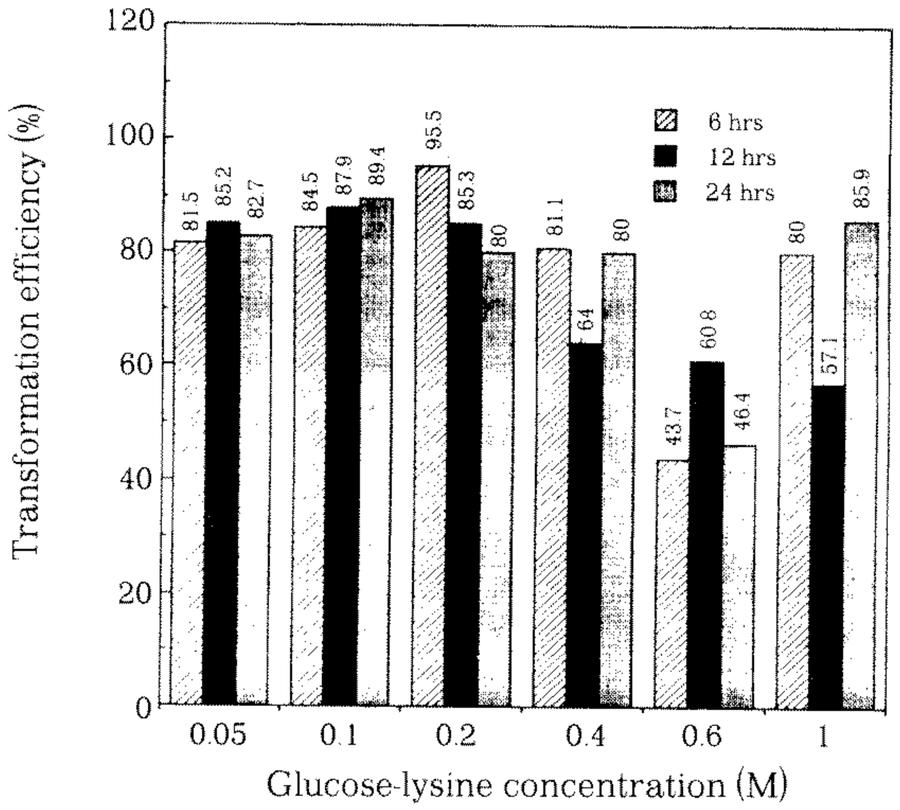

Figure 6. Transformation efficiencies of pUC12 of the reprecipitated incubation mixtures of pUC12 and different concentrations of glucose-lysine MRPs for 6, 12 and 24 hours.

ed with 0.05M, 0.1M and 0.2M glucose-lysine MRPs and incubated for 6, 12, and 24 hours were all higher than 80% (Fig 6). Those reacted with 0.4M glucose-lysine MRPs were 81.1%, 64% and 80%, respectively, while those with 0.6M glucose-lysine MRPs were 43.7%, 60.8% and 46.4%. The 1.0M glucose-lysine MRPs yielded transformation efficiencies of 80%, 57.1% and 85.9%. Transformation efficiencies did not increase as an integral function of time or concentrations of glucose and lysine incubation mixtures (Fig 6). However, an increase in more than ten-folds in transformation efficiency of the reprecipitated incubation mixtures as compared to those of the non-reprecipitated incubation mixtures was observed as shown in Figures 3 and 4.

IV. *Transformation Efficiencies of the Incubation Mixtures of pUC12 and Proteins*

MRPs of 1M glucose and 10mg/ml of BSA, lens crystallin protein, lysozyme or 1M lysine incubated for two months were obtained individually and abbreviated as GBSAMRPs, GLCPMRPs, GLYMRPs and GLMRPs, respectively. These MRPs were incubated with pUC12 for four and twelve days. The incubation mix-

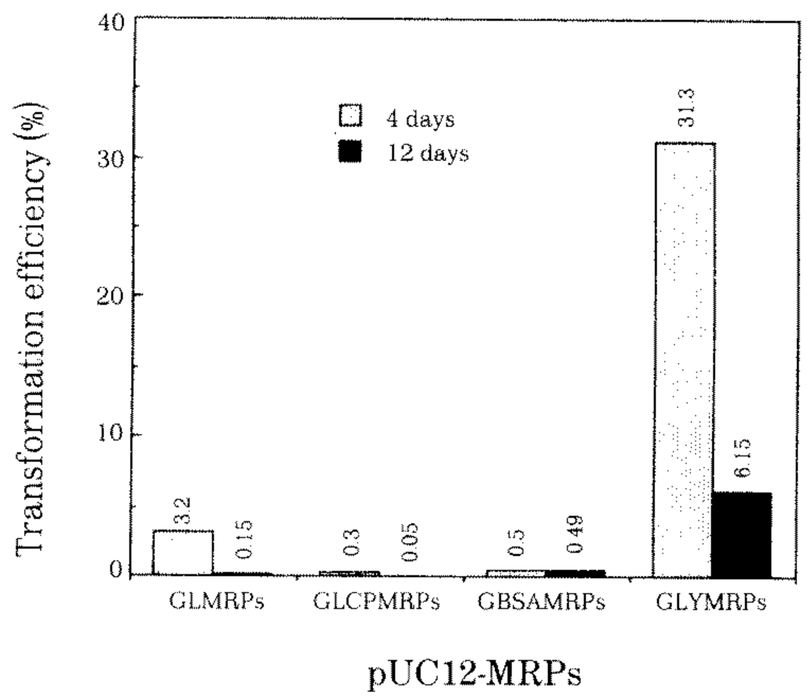

Figure 7. Transformation efficiencies of pUC12 incubated with glucose-lysine MRPs or with proteins MRPs for four days and twelve days. Incubation mixtures were subjected to direct transformation. Glucose-lysine MRPs, glucose-lens crystallin protein MRPs, glucose-BSA MRPs and glucose-lysozyme MRPs are abbreviated as GLMRPs, GLCPMRPs, GBSAMRPs and GLYMRPs, respectively.

tures were directly subjected to transformation or were reprecipitated with 70% ethanol prior to transformation. Figure 7 indicates the transformation efficiencies of the incubation mixtures without reprecipitation. Considerably lower transformation efficiencies were found in the incubation mixtures of pUC12 and GLCPMRPs or pUC12 and GBSAMRPs incubated for four and twelve days. GLCPMRPs yielded only 0.3%, 0.05%

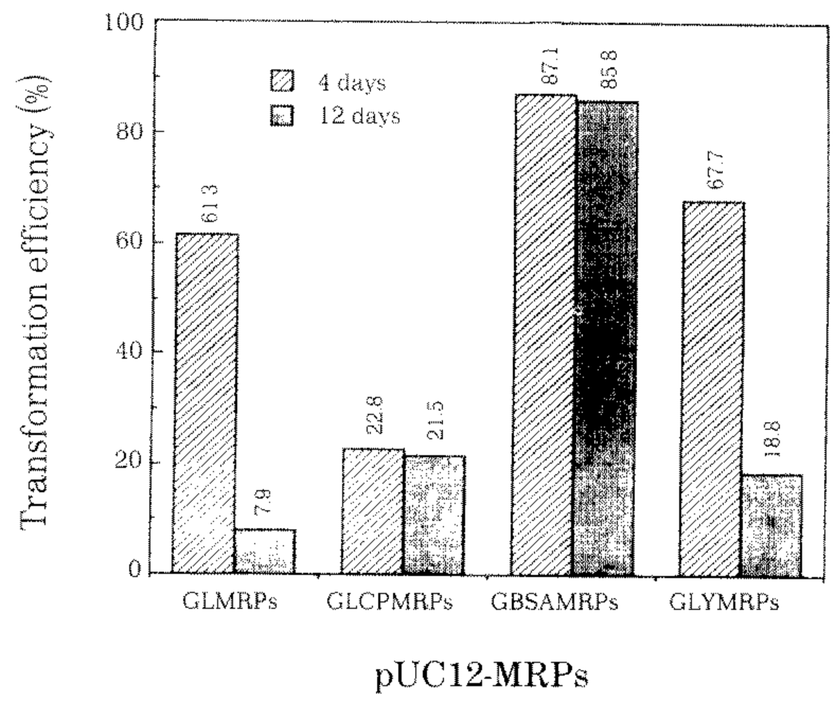

Figure 8. Transformation efficiencies of pUC12 after reprecipitation of the incubation mixtures of pUC12 and different kinds of MRPs (as given in Figure 7) incubated for four and twelve days, respectively.

while GBSAMRPs yielded efficiencies of 0.5% and 0.49%. Higher transformation efficiencies were found in pUC12 incubated with GLYMRPs, which yielded 31.3% and 6.15% for four and twelve days of incubation. For those of the pUC12 incubated with GLMRPs, transformatoin efficiencies were 3.2% and 0.15%, respectively.

Figure 8 shows the transformation efficiencies of the reprecipitated incubation mixtures of pUC12 and GLCPMRPs, GBSAMRPs, GLYMRPS or GLMRPs. Following four days incubation, they yielded 22.8%, 87.1%, 67.7% and 61.3%. After twelve days incubation, the yields were 21.5%, 85.8%, 18.8% and 7.9%. Transformation efficiencies in the incubation mixtures without reprecipitation shown in Figure 7 were lower than the reprecipitated incubation mixtures shown in Figure 8. Incubation of pUC12 with GLYMRPs and GLMRPs for twelve days were several folds lower than those incubated for four days. However, no significant difference was found in the incubation mixtures of pUC12 and GLCPMRPs or GBSAMRPs.

DISCUSSION

We have shown that MRPs could react rapidly (within in minutes) with DNA by decreasing the transformation efficiency of DNA as indicated in Figures 3 and 4. The incubation of pUC12 and lower concentrations of glucose-lysine MRPs for five minutes influences the transformation efficiency of pUC12. Even with the increase in incubation time up to twenty four hours or an increase in the concentrations of glucose-lysine MRPs did not effect the lower transformation efficiency of pUC12. It is assumed 0.025M glucose-lysine MRPs already bound to and saturated pUC12 molecule. Thus, increasing concentrations of glucose and lysine to 1M, resulted MRPs that did not influence the binding to pUC12 molecule. After incubated with the MRPs, the pUC12 DNA molecule did not show significant size difference as evidenced by agarose gel electrophoresis in Figure 5. It is assumed that the molecule of MRPs bound to pUC12 could not affect the mobility of pUC12 molecule. The expression of *lacZ* gene in pUC12 was also used as a marker for pUC12 aberration after incubation with MRPs. Several transformants were isolated and showed *lacZ* to be functionless, but the mutation reverted after several regenerations (data not shown).

When incubation mixtures of pUC12 and MRPs were denatured in 70% ethanol and renatured in TE buffer, the transformation efficiencies of the renatured DNA (Figure 6) were more than ten times of those without the reprecipitation (Figures 3 and 4). This phenomenon was also seen in the pUC12 incubated with glucose-protein MRPs (Figures 7 and 8), where transformation efficiencies were more than seventy folds (Figure 8 compared to Figure 7) for pUC12 incubated with GLCPMRPs and GBSAMRPs. As shown in Figures 8 and 7, pUC12 incubated with GLYMRPs was two folds greater (Figure 8 compared to Figure 7). It was proposed that after denaturation, the pUC12 and MRPs incubation mixtures might release the binding of the MRPs from the DNA molecule, and eliminate the interaction of MRPs with the pUC12 molecule. Thus, the transformation efficiencies of the renatured pUC12 were increased.

Transformation efficiencies of the pUC12 incubated with MRPs for twelve days were lower than those incubated for four days (Figure 7). For denaturation of the pUC12 incubated with MRPs, the transformation efficiencies of pUC12 were all increased with both incubation periods (Figure 8). However, there were exceptions; transformation efficiency of pUC12 after twelve days incubation with GLYMRPs and GLMRPs indicated lower transformation efficiencies (18.8% and 7.9%) as compared to those of four-day incubation (67.7% and 61.3%). It was assumed that longer incubation of pUC12 with MRPs might affect the structure or function of DNA , and thus could not be recovered by DNA denaturation.

The present work demonstrates that glucose-lysine or glucose-protein MRPs can interact with DNA molecules and affect the function of trans-

formation. These experiments suggest that the nonenzymatic reaction *in vivo* of sugar adducts with DNA directly or through protein crosslinks may adversely affect the function of DNA and possibly account for DNA mutations noted during the process of aging[8,14].

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梅納褐變反應產物誘導DNA變異之研究

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摘 要

本實驗利用 *in vitro* 製備之梅納褐變反應產物(MRPs)與質體pUC12分子反應,在濃度0.025M至1M之葡萄糖及離胺酸MRPs,其與pUC12之反應液於反應時間5分鐘至24小時內,皆會導致pUC12之轉形功能下降。檢視反應24小時內MRPs與pUC12反應液裡的pUC12 DNA電泳圖譜,並未發現任何DNA圖譜型式的改變。

延長MRPs與pUC12之反應時間至4天及12天,反應液裡DNA之轉形效率分別為3.2%及0.15%,反應12天之反應液裡pUC12之轉形效率明顯下降.此情形亦發生於生物蛋白lens-crystallin protein及lysozyme分別與葡萄糖反應二個月之MRPs,其與pUC12反應4天及12天後pUC12之轉形效率,反應12天者較之反應4天者低數倍。

此些MRPs與pUC12反應液經酒精變性處理並復溶於TE緩衝液,則反應液中pUC12之轉形效率較之不經酒精變性處理者增加數十倍,此情形亦見於反應不超過24小時的葡萄糖及離胺酸MRPs與pUC12之反應液。

關鍵詞：梅納反應，DNA變異，轉形效率。