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페닐글리옥살에 의한 구아닌의 화학적 변형

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Chemical Modification of Guanine with Phenylglyoxal

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요 약 페닐글리옥살과 구아닌 사이의 부가생성물을 얻었다. 그 구조는 글리옥살-구아닌 부가생성물[1]과 유사하다. 페닐글리옥살의 알데히드기는 구아닌의 1-N 위치에, 케토기는 구아닌의 N^2 위치에 부가된다. 부가생성물의 구조는 질량분광법, 핵자기공명분광법 및 과요오드산염 산화법에 의하여 결정하였다. 부가생성물을 과요오드산염으로 산화하여 N^2-벤조일-구아닌을 얻었다. 이 결과에 근거하여 구조식 I과 같이 구조를 밝혔다.

부가생성물은 알카리용액에서 안정하다. pH 12, 60 °C에서 2시간을 가열하여도 페닐글리옥살과 구아닌으로 해리되지 않는다. 부가생성물은 산성 에탄올에 녹지만 중성 또는 알카리성 물에는 녹지 않는다. 이 물질은 구아닌의 A_{280}/A_{260}의 값에 비하여 낮은 값을 가진다.

Abstract: The adduct between phenylglyoxal and guanine was prepared. The structure of the adduct is similar to the glyoxal-guanine adduct[1]. Aldehyde group of phenylglyoxal is added to 1-N of guanine base, and keto group is added to N^2 of guanine base. The structure of the adduct was determined by mass spectrometry, nuclear magnetic resonance spectroscopy and periodate oxidation. Periodate oxidation produced N^2-benzoyl-guanine from the adduct. On the basis of these results, it has been assigned the structure I.

The adduct is stable in alkaline solution: It does not dissociate into phenylglyoxal and guanine even after 2 hours heating at 60 °C at pH 12. The adduct is soluble in acidic ethanol, and is slightly soluble in neutral or alkaline water. It has a lower A_{280}/A_{260} ratio at pH 1 compared to that of guanine.

Introduction

Modification approaches to the biological function and physical properties of nucleic acids have been applied intensively, in particular, to tRNAs. Dicarbonyl compounds, such as, glyoxal, β-ethoxy-α-ketobutyraldehyde, ninhydrin and so forth have been employed by many workers for the specific modification of guanine base of tRNAs.

Shapiro and Hachmann[1] and Shapiro et al.[2] have studied the reaction between guanine and

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phenylglyoxal, but they have not given any suggestion on the structure of the addition product. No further attention has been directed on the chemical properties of the adduct and the usability of phenylglyoxalation on the study of structure and biological function of tRNAs. The preliminary studies[3,4] suggest that phenylglyoxalation of tRNAs even in small extent alters remarkably its amino aoid acceptor activity and physical properties. Phenylglyoxal may be a good agent for the chemical modification of tRNAs.

In this work we have determined the chemical structure of the adduct formed between phenylglyoxal and guanine. The addition sites of aldehyde group and keto group to guanine base has been a problem unsolved until now.

Materials and Methods

Reaction between phenylglyoxal and guanine. Guanine was purchased from Wako Pure Chemical Industry, Osaka, Japan. It was chromatographically and spectrophotometrically pure, and used without recrystallization. Phenylglyoxal monohydrate (Aldrich Chemical Co., Inc., Milwaukee, Wis., U.S.A.) was kindly donated by Dr. Dong Han Kim, Wyeth Laboratories, Inc., Phila., U.S.A., its purity indicated was 97%, it had no ultraviolet absorbing impurities and used as such.

The reaction mixture contained 100 ml of water, 0.5 ml of glacial acetic acid, 151 mg (1mmole) of guanine and 1.521 g (10.0mmole) of phenylglyoxal. The mixture was incubated for 3 days at 60 °C while it was kept stirring. After the reaction the mixture was evaporated to dryness under vacuum at 60 °C. From the solid material thus obtained the excess phenylglyoxal was removed by extracting several times with ether. A yellow substance was resulted in an amount of about 200 mg. A portion of the adduct was dissolved in acidic ethanol (1 conc. HCl : 50 absolute ethanol, v/v).

Purification of the adduct. Whatmann No. 1 paper was used for the separation of a small amount of the adduct. The paper was imbibed with ammonium sulfate and dried before use[5]. The solvent system used was ethanol-water (80: 20 v/v). The paper was allowed to run for 16 to 18 hours at room temperature by descending method. The spot of adduct was cut off and chipped into small pieces, and 0.1 *N* hydrochloride solution was used for the extraction of the adduct.

Purification of the adduct in a large scale was carried out by ion exchange column chromatography using Dowex AG 50W-X4, 200−400 mesh. The column size was 8.5×0.6 cm. 8 mg of the sample was applied on the column. The adduct was separated by eluting with 2 *N* hydrochloride solution at a flow rate of 0.6 ml/min. The fraction size was 4.5 ml. The fractions of the adduct were pooled and dried under vacuum at 60 °C. The purity was checked by paper chromatography.

Spectral analysis of the adduct. Ultraviolet absorption spectra were run on a Hitachi Model 124 Spectrophotometer, Hitachi, Ltd. Absorption spectra were controlled in aqueous solution at various pH values. The nuclear magnetic resonance (nmr) spectra were run on a Varian Associates Model V-3521A spectrometer. Adduct was dissolved in D_2O by heating for several minutes at 100 °C. Mass spectra were run on a mass spectrometer, Finnigan Model 1015.

Periodate oxidation of the adduct. Periodate oxidation of the adduct was carried out according to the method of Shapiro et al.[2]. The reaction mixture contained 26ml of water, 8 ml of ethanol, 101.8 mg adduct and 455.5 mg of sodium metaperiodate. The suspension was continuously stirred in the dark for 18 hours at 15 °C. The

reaction mixture was evaporated to dryness under vacuum at room temperature and the residue was extracted with 30 ml of hot isopropanol. The extract was filtered while hot, and its filtrate was separated by two dimensional paper chromatography. Following solvent system were used: Ethanol-water (80 : 20 v/v) for the first dimension and saturated ammonium sulfate solution isopropanol (80 : 2 v/v) for the second dimension. The paper was allowed to run 14 hours for the first dimension and 12 hours for the second dimension. N^2-benzoylguanine formed was indentified by referring to the ultraviolet spectra of the authentic compound[2].

Results and Discussion

Reaction between phenylglyoxal and guanine. The reaction was very slow. Usually 3 or 4 days were needed to have 30 % yield of the adduct. A new ultraviolet absorbing substance was produced. Exact kinetics was not studied. Shapiro et al.[2] have attempted the reaction between phenylglyoxal and guanine at pH 7, and they have reported that there occurred no reaction product even after a week's reaction. It seems that guanine did not dissolve in a solution of neutral pH.

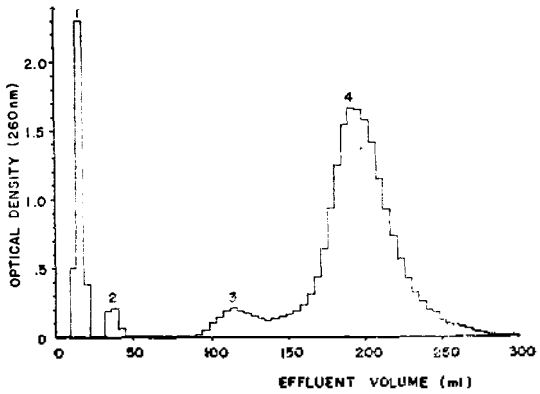


Fig. 1 Elution profile of reaction product between phenylglyoxal and guanine. 8 mg of the sample was chromatographed on 8.5×0.6 cm column of Dowex 50W—X4. The column was eluted with 2 *N* HCl. Flow rate was 0.6 ml/min. Fraction volume was 4.5 ml.

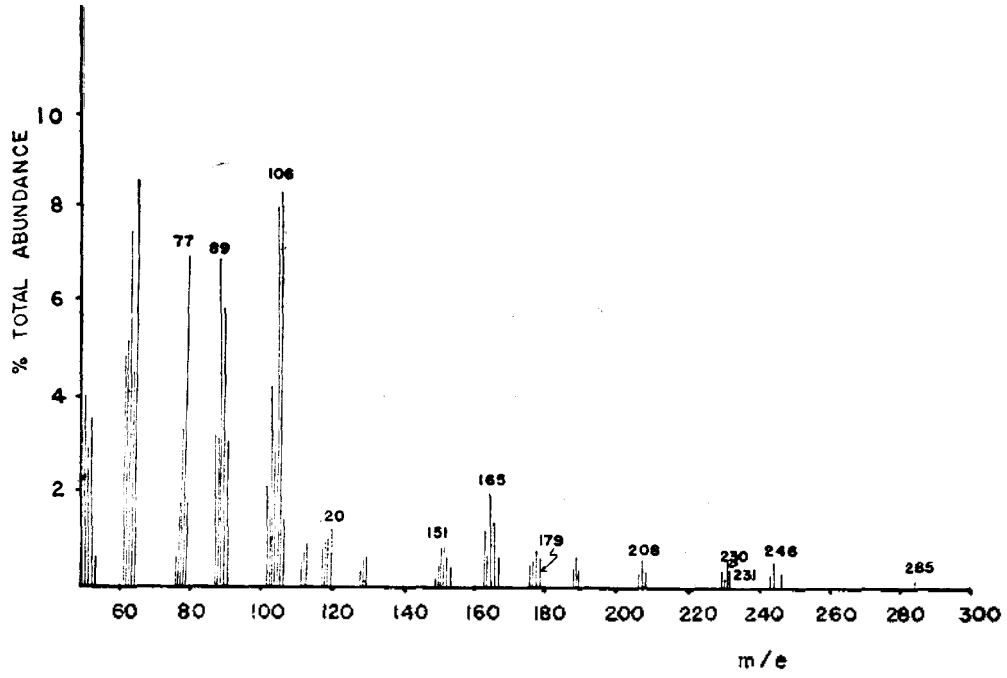
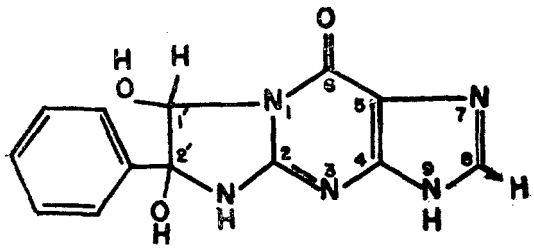


Fig. 2. Mass spectrum of the adduct. Direct inlet, 350 °C, 70 eV.

Separation of the adduct by column chromatography. The adduct was separated as mentioned in Materials and Methods. Elution profile is shown in *Fig.* 1. Peak 4 contained the only adduct formed. Peak 3 contained residual guanine, peak 1 contained excess phenylglyoxal. Peak 2 contained an unknown substance. The fast mobility through the coumn of this substance allows us to believe that it is not a purine derivative. The fractions from 170 ml to 220 ml of peak 4 were pooled and used for chemical analysis.

Determination of structure of the adduct. The mass spectra of the adduct (*Fig.* 2) shows a parent peak at m/e 285 which is equal to the mass of the adduct.



Structure I

Assuming that molecular ion for adduct would have an m/e of 285, and since guanine and its derivatives fragment into low masses[6], the following m/e assignments can be made: 77 ($-C_6H_5$); 89 ($-\overset{|}{\underset{|}{C}}-C_6H_5$); 106 ($-\overset{|}{C}(OH)C_6H_5$); 120 ($-\overset{|}{\underset{|}{N}}-C(OH)C_6H_5$); 165 {loss of 120 ($-\overset{|}{\underset{|}{N}}-C(OH)C_6H_5$)}; 179 (loss of 106 ($-C(OH)C_6H_5$)); 208 {loss of 77 ($-C_6H_5$)}; 231 {loss of 54 ($=N^3-C^4-\underset{H}{N^9}-C^8-H$)}; 246 {loss of 39 ($-\underset{\|}{C^5}-N^7=\underset{|}{C^8}-H$)}. Fragments of m/e 120 and 165 might be resulted from the possible structure occurring by the rearrangement of one H of N^2 to N-3 of the adduct. The fragmentation of the adduct suggests that the keto group is added to N^2 of guanine base. A possible structure is shown as structure I.

The most conclusive evidence about the structure of the adduct was obtained from the nuclear magnetic resonance spectra in D_2O (*Fig.* 3). The adduct showed two peaks at τ 2.39, and another at τ 4.28. The absorption at τ 2.39 was occurred by singlet five benzene protons, and another one at τ 4.28 was occurred by singlet one hemiacetal proton in the phenylglyoxal moiety of the adduct (See structure I). There was no absorption at τ 1.9 which could be occurred by H-8 of guanine. Disappearance of the absorption at τ 1.9 is caused by the exchange of H-8 of guanine with deuterium. One H-N^2, one H-N^9, one H of hydroxyl at C-1′, and one H of hydroxyl at C-2′ were exchanged with deuterium. It was shown by other workers (7, 8) that nearly complete deuteration at C-8 of adenine and guanine was obtained by heating 2 hours at 90 °C in D_2O. It was also shown that H-8 of guanine was exchanged more rapidly than H-8 of adenine. Our experiment shows that H-8 of phenylgly-

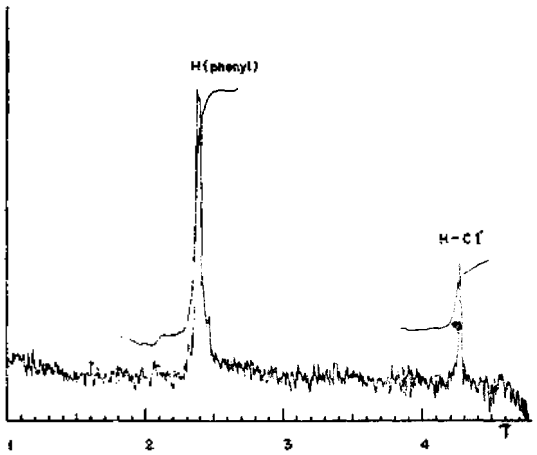


Fig. 3. The nmr spectrum of phenylglyoxal-guanine adduct in D_2O.

oxal-guanine adduct can be completely exchanged by heating only several minutes, enough time for the dissolution of the adduct in D_2O at 100 °C.

Either mass spectra or nuclear magnetic resonance spectra are not sufficient for the assignment of phenyl group position. The key to the proof of the structure (see Structure I) was the cleavage reaction of the adduct with periodate. Oxidation of the adduct by periodate proceeded smoothly, and led to the formation of N^2-benzoylguanine. Its ultraviolet spectra are shown in *Fig.* 4 and *Table* 1.

On the basis of mass spectra, nuclear magnetic resonance spectra, and finally obtaining N^2-benzoylguanine by periodate oxidation of adduct, we have determined the structure of phenylglyoxal-guanine adduct as drawn in Structure I.

Properties of the adduct. The ultraviolet spectra of the adduct are shown in *Fig.* 5 and *Table* 2. Maximum absorption and minimum absorption at pH 1 are shifted to the longer wavelength compared to that of guanine. A distinct lowering of A_{280}/A_{260} ratio at pH 1 is a useful characteristics for the spectral analysis of the adduct. The value of this ratio is 0.59 for the adduct, while that of guanine is 0.84. The adduct is stable in alkaline pH range. It did not dissociate into the phenylglyoxal and

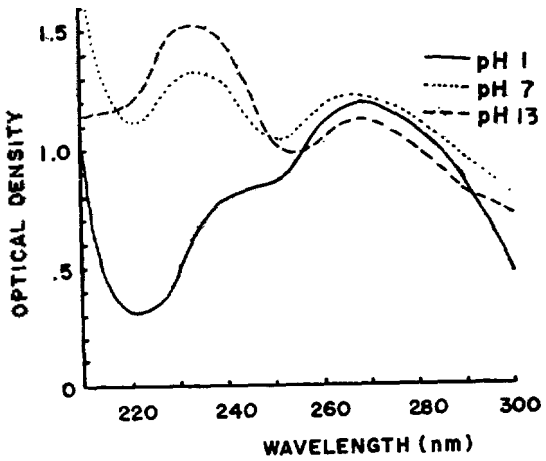


Fig. 4. The spectra of N^2-benzoylguanine.

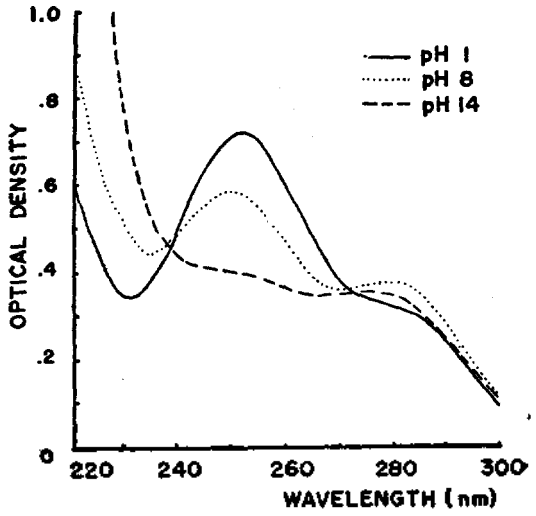


Fig. 5. The spectra of phenylglyoxal-guanine adduct.

Table 1. Spectral properties of N^2-benzoylguanine.

	pH 1	pH 5	pH 7	pH 10	pH 13
λ_{max} (nm)	268	235, 264	235, 267	233, 267	234, 268
λ_{min} (nm)	221	222, 245	221, 251	220, 255	255
A_{240}/A_{260}	0.78	0.89	1.08	1.39	1.38
A_{250}/A_{260}	0.86	0.89	0.91	1.03	1.00
A_{270}/A_{260}	1.02	0.96	1.02	1.02	1.07
A_{280}/A_{260}	0.95	0.87	0.94	0.88	0.96
A_{290}/A_{260}	0.76	0.81	0.81	0.69	0.78

Table. 2. Spectral properties of phenylglyoxal-guanine adduct.

	pH 1	pH 8	pH 13
λ_{max} (nm)	251	249, 279	252, 275
max $\times 10^{-3}$	2.0	.....	.....
λ_{min} (nm)	229	232	.....
A_{240}/A_{260}	0.91	1.10	1.13
A_{250}/A_{260}	1.30	1.30	1.05
A_{270}/A_{260}	0.65	0.79	0.99
A_{280}/A_{260}	0.59	0.82	0.97
A_{290}/A_{260}	0.39	0.58	0.64

guanine even after two hours heating at 60 °C at pH 12. Glyoxal-guanine adduct [1,9,10,11] and ketoxal-guanine adduct [12,13] were reported to dissociate into the dicarbonyl compounds and guanine at pH values higher than 7.2.

The adduct is insoluble in ether and acetone and slightly soluble in neutral or alkaline water, while it is easily soluble in ethanol-acidic water (50:1 v/v) mixture. The R_f value of the adduct in ethanol-water (80 : 20) solvent system was 0.68.

Acknowledgement

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