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REACTION OF PHENYLGLYOXAL WITH ARGININE. THE EFFECT OF BUFFERS AND pH

Shu-Tong Cheung and Margaret L. Fonda

Department of Biochemistry, Health Sciences Center,
University of Louisville, Louisville, Kentucky 40232

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SUMMARY

Phenylglyoxal reacts much more rapidly with N^2-acetylarginine than with either N^2-acetyllysine or N-acetylcysteine. The rate of the reaction of phenylglyoxal with either N-acetylarginine or arginine increases with increasing pH from 7.5 to 11.5. The model reaction with arginine is much faster in bicarbonate, diethylamine, or triethylamine buffer than in N-ethylmorpholine, borate, phosphate, or Tris buffer. This activation by various buffers should be taken into consideration when glyoxal derivatives are used to modify arginyl residues.

INTRODUCTION

Phenylglyoxal, glyoxal, and methylglyoxal have been used to specifically modify arginyl residues in a number of proteins. Takahashi (1, 2) studied model reactions of phenylglyoxal with free amino acids and N^2-dinitrophenyl amino acids. He found that phenylglyoxal most rapidly modified the guanidinium group of arginine, but also slowly deaminated α-amino acids. The product of the reaction of phenylglyoxal with arginine or N-acetylarginine was shown to contain two phenylglyoxal moieties per guanidinium group (1, 2). However, the structure of this product and the mechanism of the reaction have not been elucidated. We have shown that the rate of inactivation of glutamate apodecarboxylase by phenylglyoxal was dependent on pH, was faster in bicarbonate buffer than in other buffers, and was dependent on the concentration of the bicarbonate (3). Since phenylglyoxal is frequently used to modify arginyl residues in proteins and since we found that bicarbonate enhanced the rate of inactivation of glutamate apodecarboxylase by phenylglyoxal (3), the effects of bicarbonate and pH on the reaction between phenylglyoxal and N-acetylarginine were investigated.

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MATERIALS AND METHODS

Materials. Glyoxal, methylglyoxal, phenylglyoxal, 2,3-butanedione, L-arginine, N^2-acetyl-L-arginine, N^2-acetyl-L-lysine, and N-acetyl-L-cysteine were purchased from Sigma Chemical Co. All buffers were prepared with reagent grade chemicals in double-distilled water. Measurements of pH were made at 23°C with a Radiometer model 28 pH meter.

Reaction of phenylglyoxal with N-acetyllysine or N-acetylcysteine. Reaction mixtures containing 25 mM phenylglyoxal and 5 mM of either N^2-acetyllysine or N-acetylcysteine in sodium bicarbonate, pH 7.5, were incubated at 23°C for 1 to 4 days. The amount of remaining N-acetyl amino acid was determined by its reaction with recrystallized 2,4,6-trinitrobenzenesulfonic acid (4).

Reaction of phenylglyoxal with arginine or N^2-acetyl-L-arginine. Reaction mixtures containing 25 mM phenylglyoxal and 5 mM N^2-acetyl-L-arginine in the buffer designated were incubated at 23°C for approximately 60 min. The reaction was monitored by following the increase in absorbance at 340 nm (1) with a Zeiss PMQ II spectrophotometer equipped with a temperature-controlled cuvette holder. Buffer containing 25 mM phenylglyoxal was used as a blank. All absorption spectra were obtained with a Cary 14 spectrophotometer.

The reaction between 25 mM phenylglyoxal and 5 mM L-arginine was followed either by monitoring the loss of guanidinium groups reacting in the Sakaguchi test (5) or by amino acid analysis. At various times, 80-µl aliquots of the reaction mixture were removed, diluted into 320 µl of sodium citrate, pH 2.2, and stored frozen until amino acid analysis by a single column procedure described by Benson (6). Aliquots (100 µl) were also removed from the same reaction mixture and diluted into 2.4 ml of water for the Sakaguchi reaction, which was performed immediately. The Sakaguchi test used was a modification of the procedure described by Dubnoff (5). The excess gas was removed at reduced pressure, and the absorbance was read 1 min after mixing all reagents. Unreacted phenylglyoxal interfered somewhat with the Sakaguchi reaction (7). One mM phenylglyoxal inhibited the Sakaguchi test with 200 µM arginine approximately 25%. The data reported have been adjusted for this interference.

RESULTS

The arginine reactions in this study were followed by measuring the increase in absorbance at 340 nm, the loss of guanidinium groups reacting in the Sakaguchi test, and the loss of arginine as measured by amino acid analysis. The guanidinium group after reacting with phenylglyoxal does not give a positive Sakaguchi test.

In bicarbonate, pH 7.5, phenylglyoxal reacted rapidly with N-acetylarginine, and very slowly, if at all, with N-acetyllysine and N-acetylcysteine (Fig. 1). The same rate of reaction between phenylglyoxal and L-arginine was observed whether the reaction was followed by measuring the loss of reactive guanidinium groups in the Sakaguchi test or the loss of arginine by amino acid analysis. Therefore, the α-amino group of arginine

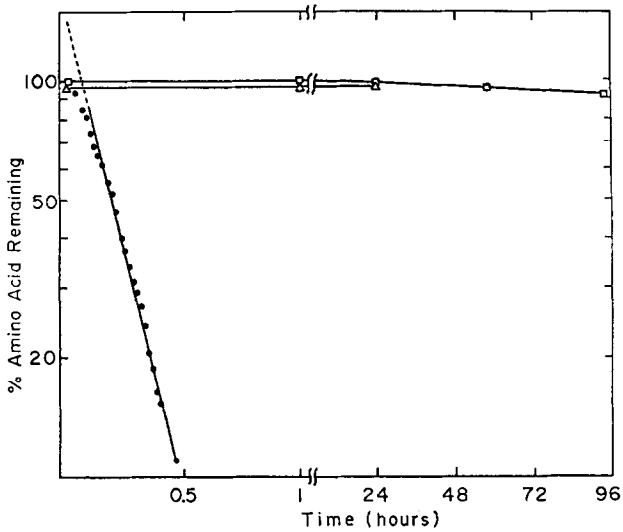

Figure 1. The rates of modification of N-acetylarginine (●), N-acetyllysine (□), and N-acetylcysteine (Δ) by phenylglyoxal in 83 mM sodium bicarbonate, pH 7.5. The reaction with N-acetylarginine was monitored by measuring the increase in absorbance at 340 nm. The amounts of unreacted N-acetyllysine and N-acetylcysteine were determined by reaction with 2,4,6-trinitrobenzenesulfonic acid.

TABLE I[a]

Effects of pH on the Reaction Rates of Arginine Reagents with L-Arginine

	Rate Constants with			
pH	Phenylglyoxal (min^{-1})	Methylglyoxal (min^{-1})	Glyoxal (min^{-1})	2,3-Butanedione (min^{-1})
7.5	0.164	0.41	0.005	0.002
8.5	0.283	0.78	0.007	0.002
9.5	0.77	2.63	NT	0.001
10.5	2.94	9.25	NT	0.002
11.5	6.08	20.8	NT	0.002

[a]The reaction mixtures contained 5 mM L-arginine and 25 mM of the arginine reagent in 83 mM bicarbonate buffer. Aliquots were removed at different time intervals to use in the Sakaguchi test. The data were plotted as log guanidinium *versus* time. The rate constants calculated from the slopes are given. NT - not tested.

was not modified significantly in this period of time. The reaction of the guanidinium group with phenylglyoxal was very specific under these conditions.

The reaction between N-acetylarginine or arginine and phenylglyoxal was carried out at various pH values in bicarbonate-carbonate buffer. The reaction rate increased with increasing pH (Table I). The same rates were obtained with N-acetylarginine as with arginine.

The rate of the reaction between phenylglyoxal and either N-acetyl-arginine or arginine was greatly affected by the type of buffer used in the reaction mixture (Fig. 2). The reaction was much faster in bicarbonate,

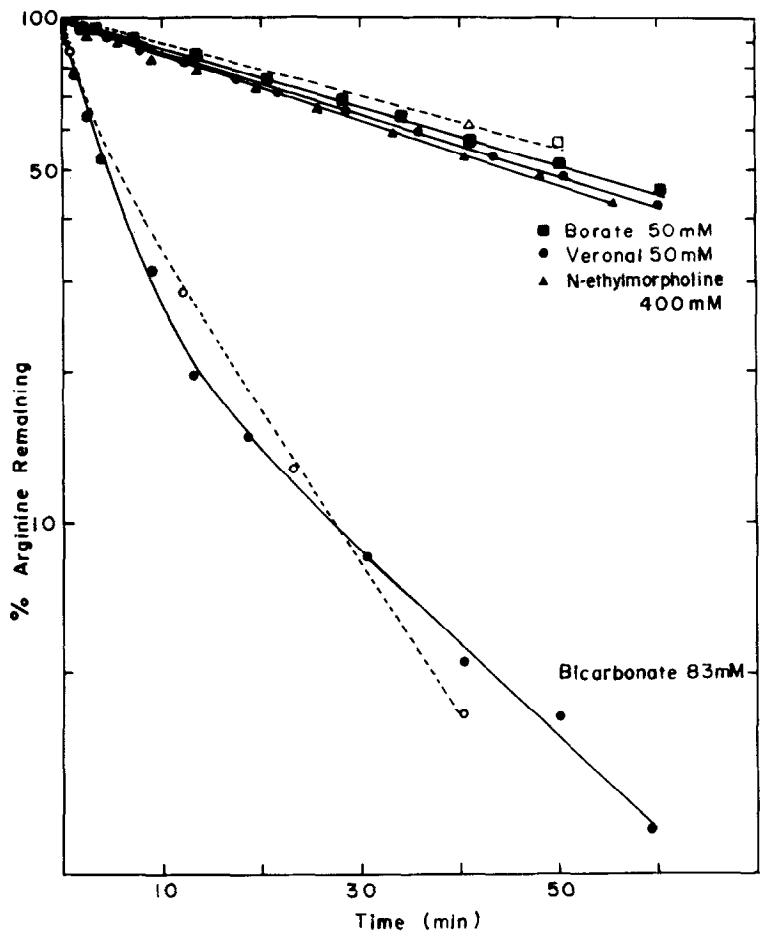

Figure 2. Reaction of arginine and phenylglyoxal in various buffers. The reaction mixtures contained 5 mM L-arginine and 25 mM phenylglyoxal in the designated buffer, pH 7.5. Aliquots were removed at various times, and arginine concentrations were determined by the Sakaguchi test (solid lines and closed symbols) or by amino acid analysis (dashed lines and open symbols).

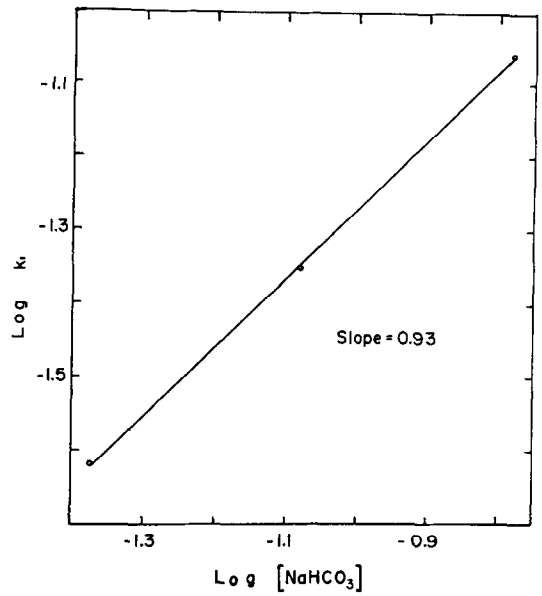

Figure 3. Plot of logarithm apparent first-order rate constants *versus* logarithm bicarbonate concentrations. The reaction mixture contained 5 mM N-acetylarginine and 25 mM phenylglyoxal in sodium bicarbonate, pH 7.5. The absorbance at 340 nm was recorded, and the rate constants were obtained from the slopes of the plots of ln $(A_\infty - A_t)$ *versus* time.

diethylamine, or triethylamine buffer than in N-ethylmorpholine, Tris, borate, phosphate, or Veronal buffer. The reaction of N-acetylarginine with phenylglyoxal in bicarbonate was greatly retarded by adding 57 mM borate into the reaction mixture.The rate of the reaction between N-acetyl-arginine and phenylglyoxal was directly proportional to the bicarbonate concentration. A plot of log rate constants *versus* log bicarbonate concentrations was a straight line with a slope of 0.93 (Fig. 3). This would indicate that the reaction is first-order with respect to bicarbonate (8).

The effect of pH and buffer on the rate of reaction of L-arginine with other arginine reagents was investigated. Table I shows the rate constants obtained at pH values from 7.5 to 11.5 in bicarbonate buffer. The rate of the reaction of methylglyoxal with L-arginine was faster than that of phenyl-glyoxal with L-arginine and also increased with increasing pH. However, under these conditions 2,3-butanedione and glyoxal reacted very slowly with L-argin-

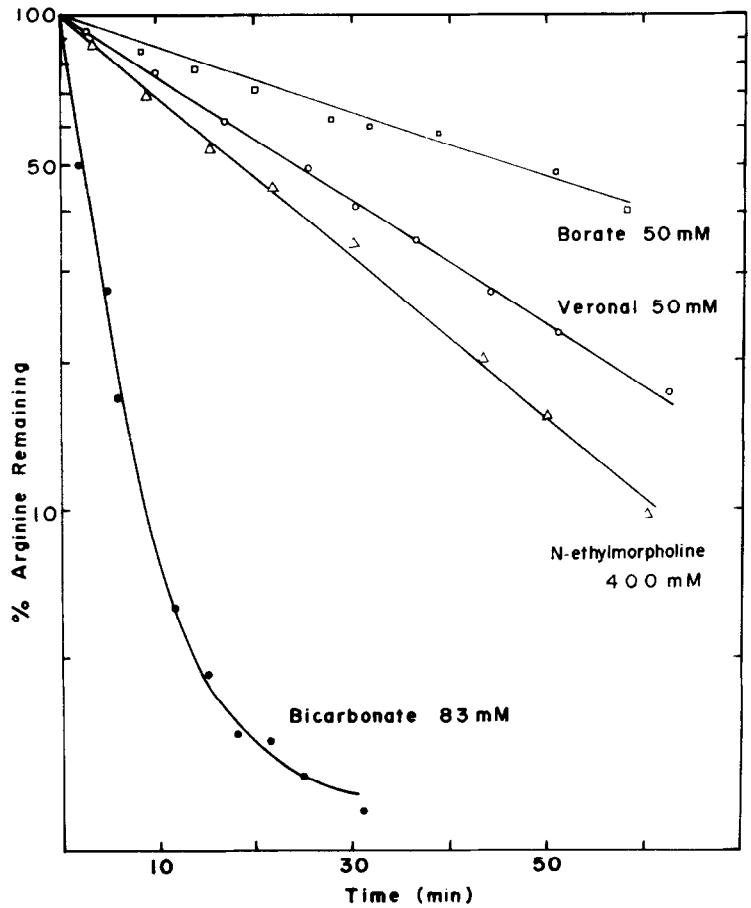

Figure 4. The effect of various buffers on the rate of reaction of L-arginine with methylglyoxal. The reaction mixtures contained 5 mM arginine and 25 mM methylglyoxal in the buffers indicated. Aliquots were removed at various times for determination of the amount of arginine remaining by the Sakaguchi test.

ine. The rate of reaction of L-arginine with methylglyoxal was much faster in bicarbonate than in N-ethylmorpholine, Veronal, or borate buffer (Fig. 4).

DISCUSSION

The rate of inactivation of glutamate apodecarboxylase by phenylglyoxal was dependent on pH, on the buffer, and on the concentration of bicarbonate buffer (3). In this study we found that the rate of the model reaction between the guanidinium group of arginine and phenylglyoxal was also dependent on pH, on the type of buffer, and on the concentration of bicarbonate.

The rate of the reaction of arginine with either phenylglyoxal or methylglyoxal increased with increasing pH. Takahashi (1, 2) also observed that the

rate of reaction of arginine with phenylglyoxal in a variety of buffers increased with increasing pH. In addition, the inactivation of enzymes in a variety of buffers by glyoxal derivatives is pH dependent (1, 3, 9-17). The pH dependency observed in the model reactions cannot be due to the ionization of amino acids other than arginine and is not due only to an effect of pH on the buffer. The observed pH dependency is due in part to the effect of pH on the ionization of arginine and/or phenylglyoxal monohydrate. The reaction between the guanidinium group and phenylglyoxal is probably a nucleophilic addition reaction, and only the non-protonated form of the guanidinium group reacts with phenylglyoxal.

Buffer greatly affected the rates of model reactions between -dicarbonyls and the guanidinium group of arginine. This buffer effect may be related to a number of factors. Complexes of phenylglyoxal with borate (1, 18) and with secondary and tertiary amines (19) have been reported. Interaction with these buffers would reduce the concentration of the reactive form of phenylglyoxal. Some of the buffers used may affect either the degree of hydration of phenylglyoxal or the ionization of one of the reagents.

Reagents that can selectively modify arginyl residues under mild conditions have been used to modify arginyl residues in many proteins that bind negatively-charged ligands (20, 21). The data presented here show that the rates of reaction of the guanidinium group of arginine with phenylglyoxal or methylglyoxal are enhanced greatly by certain buffers, including bicarbonate. Because of this rate enhancement, protein arginyl residues can be modified in these buffers by incubation with lower concentrations of phenylglyoxal for shorter periods of time. Thus, the modification of arginyl residues may be more selective under these conditions.

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