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Exploring Arylglyoxals as the Arginine Reactivity Probes. A Mechanistic Investigation Using the Buffer and Substituent Effects

KUNNUMAL BABURAJ AND SUSHEEL DURANI[1]

The Biosciences and Engineering Group, Department of Chemistry, Indian Institute of Technology, Powai, Bombay 400 076, India

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The modification of arginine side chain by the α-dicarbonyl reagents was submitted to a mechanistic investigation. The reactivity of 4-substituted phenylglyoxals toward N_α-acetylarginine was found to be dependent on the substituent as well as buffer effects. Borate formed a complex with the glyoxal–hydrates to cause an activation of the electron-rich glyoxals and an inactivation of the electron-deficient glyoxals. The ρ value in the Hammett correlation in this buffer was found to be -1.0. Bicarbonate also complexed with the glyoxal–hydrates, to increase their reactivity irrespective of the substituent effects. The Hammett correlation in this buffer gave the ρ value of $+1.0$. On the basis of these and related observations, the mechanism in arginine modification has been formulated as a sequential process involving the intermolecular attack of guanidine at the α-dicarbonyl, the pH-dependent deprotonation of the resulting monocarbinolamine, and the intramolecular bond formation within this intermediate as the rate-determining step in the overall reaction.

INTRODUCTION

Certain simple α-dicarbonyls, exemplified by phenylglyoxal (PGO),[2] are well-known for their remarkably selective modification of the arginyl residues in proteins (*1, 2*). The extensive usage of the reagents for over two decades has further established their remarkable attribute in often modifying only selected arginyls (*3–14*), although large numbers are invariably present in most proteins, with most being surface exposed (*15*). Particularly susceptible toward selective modification appear to be the arginyls that occur in the anion recognition centers in proteins, or in the active sites of the enzymes that act on anionic substrates (*12*). The kinase (*2, 13, 16*), the dehydrogenase (*3, 17–19*), the ATPase (*14*), the nuclease (*20*), and the carboxypeptidase (*21, 22*) active sites may be mentioned as the notable examples from among those that are known to feature the reactive arginines. Though the factors that influence the arginine reactivities in proteins remain obscure,

[1] To whom correspondence should be addressed.

[2] Abbreviations used: PGO, phenylglyoxal; HOPGO, 4-hydroxyphenylglyoxal; MeOPGO,4-methoxyphenylglyoxal; DMAPGO, 4-(*N,N*-dimethylamino)phenylglyoxal; ClPGO, 4-chlorophenylglyoxal; *N*-Ac-Arg, N_α-acetyl-L-arginine.

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positive electrostatic potential has been suspected as the causative factor for the reactive arginines in the anion recognition centers (*12*). A correlation between the functional role of an arginine and its chemical reactivity is thus implied and warrants an investigation. It should be possible to pursue such an objective through recourse to the α-dicarbonyls as the arginine-reactivity probes. In embarking on such a venture, however, better understanding of the mechanism in arginine modification becomes an imperative.

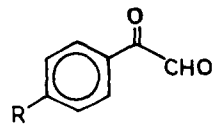
Apparently a complex process, the mechanism in arginine modification has remained an obscure one (*21, 23–27*). In its kinetic as well as chemical profile, the modification is known to be sensitive to a number of variables, including the reagents and the buffers used (*1, 2, 23*). A variety of products have either been characterized or are suspected to be formed under different conditions. It has been suggested, however, that irrespective of the eventual outcome, the first and the obligatory step in the reaction may always consist of the formation of a cyclic 1 : 1 dicarbinolamine which, depending upon the conditions, can suffer a number of alternate fates (*23*). It is quite possible that the formation of this intermediate also is rate limiting in the overall reaction, and that it dictates the kinetics in arginine modification. Two alternatives can be considered regarding the rate-determining step in the dicarbinolamine formation; (i) the intermolecular bond formation between guanidine and α-dicarbonyl or, (ii) the intramolecular cyclization of the resulting monocarbinolamine intermediate. In distinguishing between these alternatives, PGO was envisaged as the particularly relevant substrate. Given its chemically distinct carbonyl centers, a distinction between the attack at aldehyde or at ketone becomes feasible, while in doing this, the Hammett-type correlation—a number of PGO analogues being available—becomes the convenient mechanistic aid. Based on this reasoning we have undertaken a study of the substituent as well as buffer effects in the arginine reactivity of PGO, and the results are presented here.

MATERIALS AND METHODS

Materials. *N*-Ac-Arg was from Sigma Chemicals. All other chemicals were of reagent or analytical grade.

Synthesis. PGO, HOPGO, MeOPGO, and ClPGO were synthesized according to the reported procedures (*28–30*). DMAPGO was similarly synthesized via SeO_2 oxidation of *p*-(*N,N*-dimethylamino)acetophenone. It was crystallized as a hydrate from methanol–water and furnished the following data: mp 121–25°C; ir (Nujol) 1630 cm^{-1} (γ_{co}); uv (EtOH) λ 357 nm (ε 22,000 M^{-1} cm^{-1}); ^1H NMR ($CDCl_3$) δ 3.07 and 3.11 (3H, 2 s, $OHCCOC_6H_4N(C\underline{H}_3)_2$ and $(OH)_2CHCOC_6H_4N(C\underline{H}_3)_2$), 6.27 and 9.67 (1H, 2 s, $-C\underline{H}(OH)_2$ and $-C\underline{H}O$), 6.64 and 6.67 (2H, 2 d, Ar–H, *ortho* to $-COCH(OH)_2$ and $-COCHO$) and 8.02, 8.14 (2H, 2 d, Ar–H, *meta* to $-COCH(OH)_2$ and $-COCHO$); MS, *m/z* 177 (40%; M^+), 148 (100%; $M^+ - CHO$).

Spectrophotometric measurements. All spectrophotometric measurements were at 27 ± 0.1°C, in a Shimadzu UV-265 instrument fitted with a thermostated cuvette compartment, and an external bath circulator.



R = H;	PGO
R = OH;	HOPGO
R = Cl;	ClPGO
R = NMe_2;	DMAPGO
R = OMe;	MeOPGO

CHART I

Glyoxal reactions with buffers. The glyoxals (100 μM) were incubated in the indicated concentration of a buffer (pH 9.0), in dark at 27°C, and the solutions were analyzed for any time-dependent absorbance changes, or for the total absorbance change at equilibrium, which required less than about 5 min in the case of borate.

Glyoxal reactions with N-Ac-Arg. The arylglyoxals were preequilibriated in the requisite buffers, at pH 8.0 or 9.0, for 10 min. An aliquot of a stock solution of *N*-Ac-Arg in the same buffer was then added, to obtain the requisite final concentration of the amino acid. The solutions were incubated in dark, at 27°C, and analyzed spectrophotometrically, either for the time-dependent absorbance changes, or for the total absorbance change after equilibriation. The experiments involving different buffers or variable buffer concentrations were always at the constant ionic strength 0.1, which was maintained by adding NaCl. The kinetic runs, monitored by the time-dependent decrease in the reagent concentrations, were allowed to proceed to completion so as to note and correct for the product absorbance at the concerned wavelength. The completion of each run, represented by the asymptote of the initial rapid absorbance change, required about an hour in borate buffer and about 3 hr in the nonborate buffers. The k_{obs} values were calculated from the ln [unreacted reagent] vs time plots.

RESULTS

The PGO analogues analyzed here for their arginine reactivities are shown in Chart 1. The reagents were first assessed for their reactions with the buffers, if any. When incubated in a number of buffers (those shown in Table 1), only in borate was a notable absorbance change observed, suggesting the conversion of the reagents into the products that lacked the ketonic groups. A typical result illustrating the borate reaction of ClPGO is shown in Fig. 1. In increasing borate, the reagent is seen to be transformed into a product with an absorption band at 220 nm. The results in Fig. 2 reveal that the borate reactions of the PGOs also are

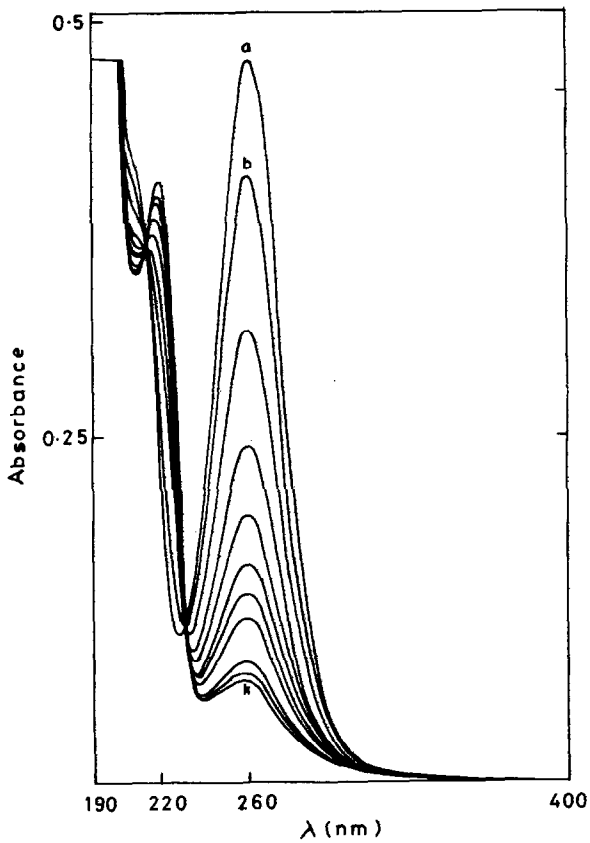


FIG. 1. Borate reaction of ClPGO. Ultraviolet absorption spectrum of ClPGO (33.3 μM) after incubation, for 10 min, in the absence (trace a) or the presence of increasing borate (3.3–75.0 mM; traces b–k), at pH 9.0.

substituent dependent. Thus, DMAPGO, an electron-rich glyoxal, is much less susceptible to the concentration dependent borate effect than is PGO, or ClPGO—an electron-deficient glyoxal.

When reacted with a single large concentration of *N*-Ac-Arg (25 mM), the PGOs (100 μM) were found to suffer a decrease in their long wavelength absorption bands. With borate as the buffer, this decrease was almost 100% of the total reagent absorbance, while in non-borate buffers, it was invariably to the extent of about 50% only. Also, as inferred from the clear isosbestics (not shown), single and stable products were found to be formed in borate, while in other buffers the reactions were accompanied by certain slower secondary absorbance changes of unknown origin, indicating the product instability. In further analyzing the buffer effects on the reaction products, the method of continuous variation (*31, 32*)—also called the Job plot—was employed. In borate, as exemplified by the result shown in Fig. 3, the Arg–PGO reaction stoichiometry was found to be 1 : 1. Although in non-borate buffers, the product instabilities did not always permit unequivocal

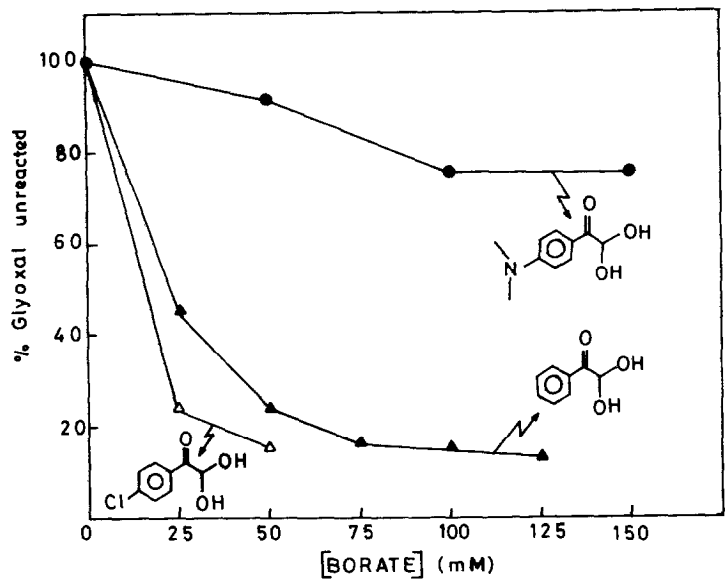


FIG. 2. Substituent effects in the borate reaction of PGO. DMAPGO, ClPGO and PGO (100 μM each) were incubated (10 min; 27°C) in the indicated concentration of borate (pH 9.0) and the solutions were analyzed for the absorbance due to the unreacted glyoxals, as illustrated in Fig. 1.

conclusions, the reaction stoichiometry of 1 : 2 was generally observed (results not shown), in agreement with the similar result reported recently by Duerksen and Wilkinson (*33*).

The reactions of PGOs (100 μM) with *N*-Ac-Arg (25 mM) were pseudo-first-order, with the k_{obs} values showing a marked sensitivity towards the substituent as well as buffer effects. The results in Fig. 4 reveal that with borate, bicine as well as bicarbonate as the buffer, the log k_{obs} values for the PGOs are linearly dependent on their substituent Hammett constants. The log k_{obs} in the case of HOPGO, in bicarbonate and bicine buffers, is, however, a notable exception in being lower than would be expected, considering the Hammett σ value -0.52 for $-O^-$ (*34*). The phenolic hydroxyl in this molecule has a pK_a of about 7.5 (*35*) and, since at pH 9.0 it would be substantially ionized, the use of the σ corresponding to $-O^-$ appears justified. Because of the deviation, HOPGO was excluded from the calculation of the least square line and its slope (ρ) in the Hammett plots in bicine and bicarbonate buffers. The ρ values in bicarbonate and bicine were found to be $+1.0$, while that in borate was -1.0.

The sensitivity of k_{obs} toward the buffer and substituent effects is further illustrated by the results summarized in Table 1. Compared to PGO, DMAPGO is less reactive in all the buffers except borate. Even bicarbonate is favorable for the reactivity of DMAPGO, but its effect is less marked than that of borate. PGO is less susceptible to the buffer effects; however, the inhibitory effect of borate and the catalytic effect of bicarbonate is evident in its reactivity.

The reactivities of DMAPGO and PGO were next analyzed for their dependence

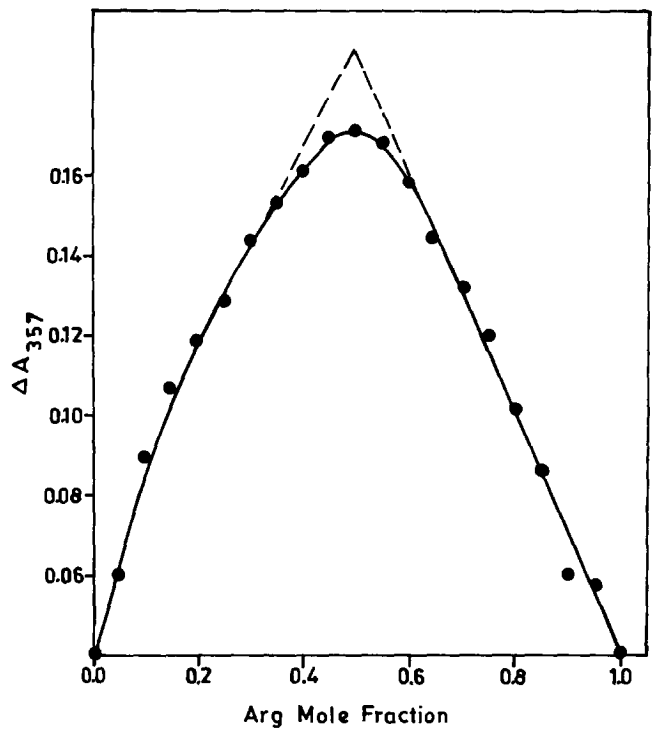


FIG. 3. Stoichiometry in the DMAPGO : *N*-Ac-Arg reaction in borate. DMAPGO and *N*-Ac-Arg were mixed in different proportions to the combined total concentration of 100 μM in borate buffer (50 mM, pH 9.0) and incubated for 12 h to ensure complete equilibriation. The absorbance values of the solutions at 357 nm were noted and subtracted from the absorbance of DMAPGO alone at equivalent concentration, after incubation in borate for the same period. The figure shows a plot of ΔA_{357} versus mole fraction of *N*-Ac-Arg.

on the buffer concentrations. In determining the borate and bicarbonate effects, bicine was used as the buffer base. The concentration dependent effect of bicine was also analyzed, with bicarbonate as the buffer base. The results are summarized in Fig. 5. Increasing bicarbonate is seen to activate both the reagents, though the effect is of a relatively small magnitude. With bicine, except for the initial sharp decrease in the reactivity of PGO, no marked concentration dependent effects are noticeable, either on PGO or on DMAPGO. The concentration dependent effect of borate is quite marked and manifests as a pronounced activation in the case of DMAPGO and an equally pronounced inactivation in the case of PGO. From the data in Fig. 6 the decrease in PGO reactivity in borate is seen to correlate linearly with the concentration of unreacted glyoxal in the given concentration of the buffer.

DISCUSSION

The nature of Arg–PGO reaction. Our results regarding the nature of products are in broad agreement with the suggested pathway (*23–27*) in the Arg-α-dicarbonyl

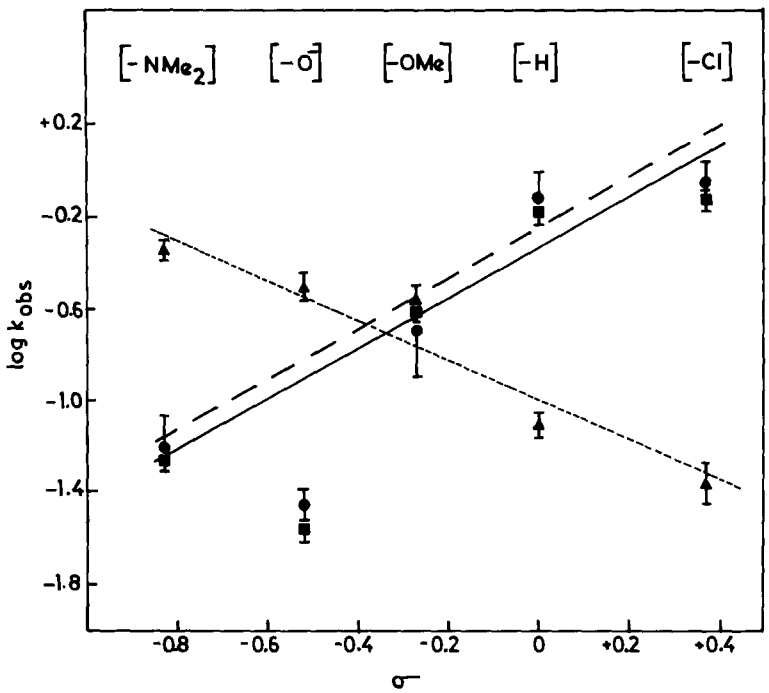


FIG. 4. Substituent effects in the arginine reactivity of PGO in borate, bicarbonate, and bicine buffers. The indicated glyoxals (100 μM each) were reacted with *N*-Ac-Arg (25 mM) in borate (▲—▲), bicarbonate (●—●) or bicine buffers (■—■) (50 mM each, pH 9.0) and the reactions were monitored as described under Materials and Methods. The data were plotted as ln (glyoxal unreacted) versus time, and the rate constants (k_{obs}) calculated as the slopes. The figure shows the plot of logk_{obs} versus the substitutent Hammett constants. Each point is mean ± SD from three independent determinations.

TABLE 1

Buffer Effects in the Arginine Reactivity of PGO and DMAPGO

Sl. No.	Buffer	PGO		DMAPGO	
		k_{obs} (min^{-1})	$t_{1/2}$ (min)	k_{obs} (min^{-1})	$t_{1/2}$ (min)
1	Bicarbonate	0.184	3.7	0.027	25.7
2	Borate	0.071	9.7	0.159	4.4
3	Bicine	0.173	4.0	0.015	46.2
4	*N*-Methylmorpholine	0.145	4.8	0.015	46.2
5	Triethanolamine	0.129	5.4	0.015	46.2
6	Hepes	0.099	7.0	0.015	46.2
7	Phosphate	0.108	6.4	0.015	46.2

Note. The reaction mixtures were 25 mM in *N*-Ac-Arg and 100 μM in PGO or DMAPGO, in the indicated buffers (50 mM) at pH 8.0, with the ionic strengths set to 0.1 with NaCl. The pseudo-first-order rate constants (k_{obs}) were determined as described in Fig. 4.

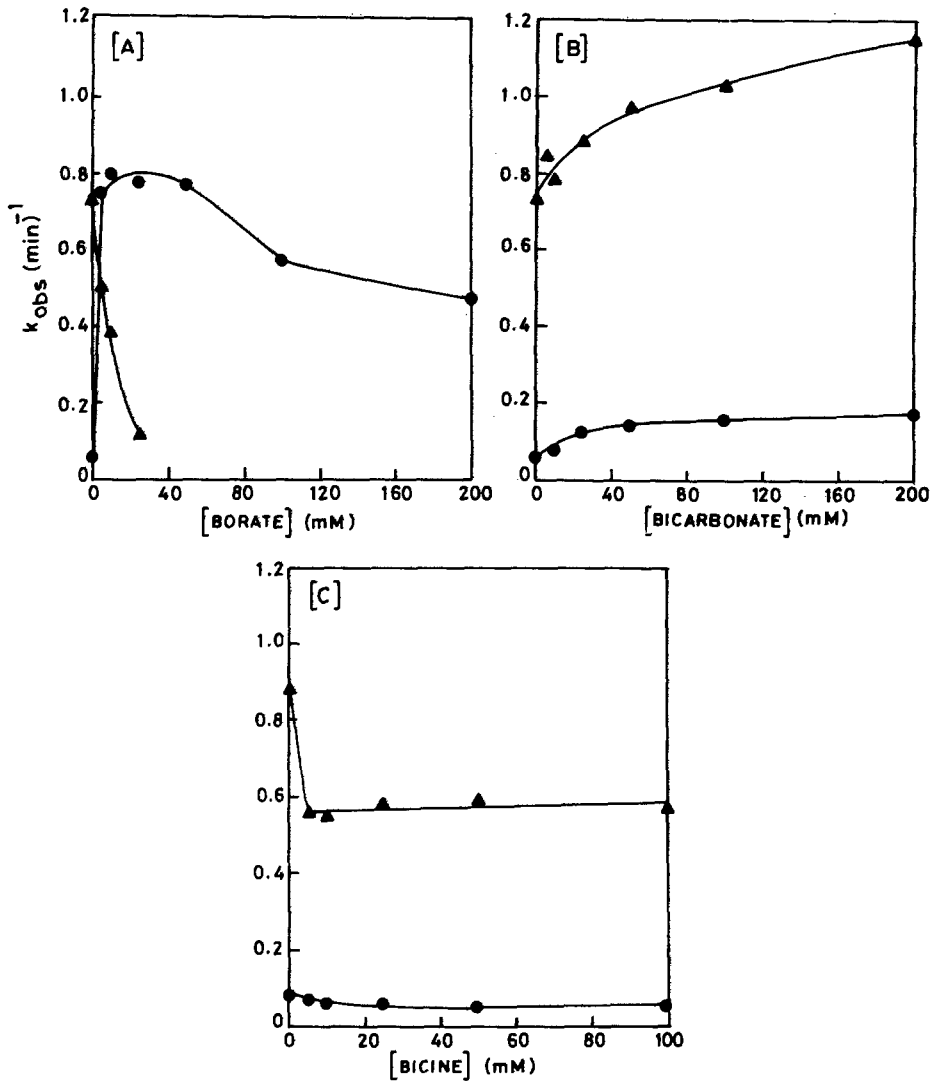


FIG. 5. Concentration-dependent buffer effects in the arginine reactivity of arylglyoxals. PGO (▲—▲) and DMAPGO (●—●) (100 μM each) in bicine (A and B) or bicarbonate buffer (C) (50 mM each; pH 9.0) were reacted with *N*-Ac-Arg (25 mM) in the presence of the indicated concentration of borate, bicarbonate, or bicine, and the k_{obs} values were determined as described in Fig. 4.

reaction shown in Scheme I. In borate, the reaction stoichiometry being 1 : 1, the product appears to be the borate-stabilized dicarbinolamine **ii** (*21, 26*). The 50% decrease in the reagent absorbance in non-borate buffers, even when *N*-Ac-Arg is in large excess, and the reaction stoichiometry of 1 : 2, agrees well with the notion that in the absence of borate the dicarbinolamine **i** stabilizes by complexation with another molecule of the reagent (*10, 25, 27*). This complexation apparently involves

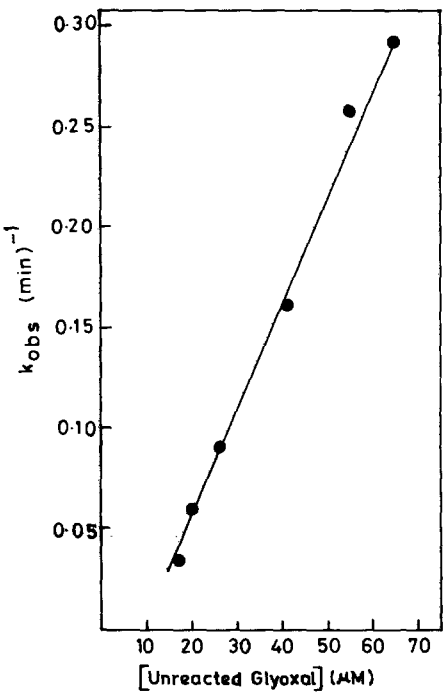
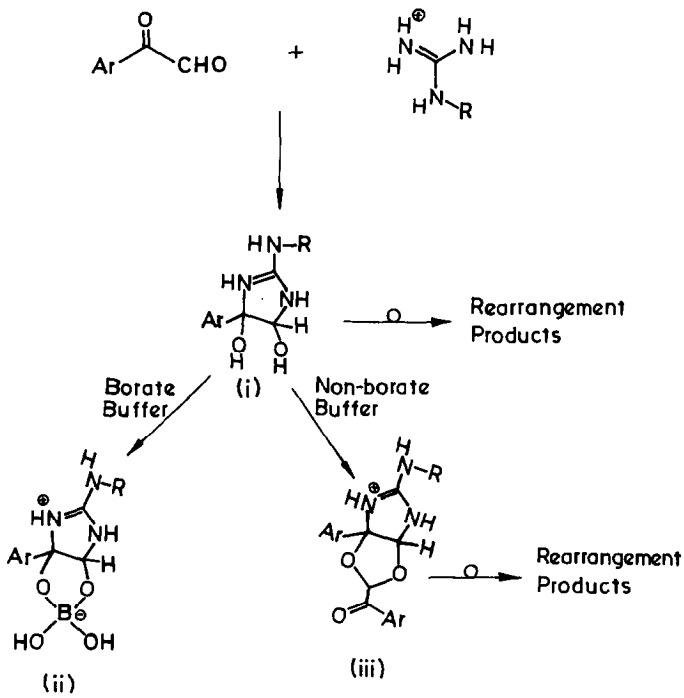


FIG. 6. Inhibitory effect of borate on the arginine reactivity of PGO. PGO was incubated in increasing concentration of borate (13–50 mM, pH 9.0) and analyzed for the residual absorbance at 251 nm. The solutions were then mixed with *N*-Ac-Arg (25 mM) and the k_{obs} values determined as described in Fig. 4. The figure shows the plot of k_{obs} versus the residual concentration of PGO after the borate reaction.

only the aldehyde group of PGO, causing the wavelength as well as the extinction coefficient of the product **iii** to be approximately equal to that of the reagent. The fact that PGO prefers to react with the dicarbinolamine rather than *N*-Ac-Arg, even when the latter is in large excess, implies first the favorable thermodynamic stability of the 1 : 2 complex, **iii**, and second, the large activation energy in the guanidine attack at the ketonic group in **iii**. The observed product instability in non-borate buffers is also in agreement with the frequent reports of rearrangement products in the Arg-α-dicarbonyl reactions (*23*). Particularly germane in the context of the present investigation is, however, the broad conclusion that it is the formation of the dicarbinolamine **i** that constitutes the initial step in the Arg-α-dicarbonyl reaction. Although in non-borate buffers the reaction stoichiometry is 1 : 2, the reaction of PGO with the dicarbinolamine intermediate makes no contribution to the absorbance changes, and hence does not figure in our kinetic experiments. Therefore the studies here, dealing with the initial PGO disappearance, only concern the formation of the dicarbinolamine and not its subsequent fate.

PGO as an electrophile. PGO is a unique α-dicarbonyl. It features a ketone which, given its resonance interaction with the aromatic ring, is a poorer electrophile than its aliphatic counterparts in cyclohexanedione and butanedione (*1*,



SCHEME I

2)—the other commonly used arginine modifiers. The aldehydic group in PGO, on the other hand, is expected to be highly electrophilic, and an inquiry into the extent of its hydration and complexation with other nucleophiles becomes important.

The partial equilibria in the PGO hydration and ionization are illustrated in Scheme IIa. The equilibrium constant (K_h) in the hydration is unknown, but may be estimated using the Hammett–Taft correlation **1,** suggested by Greenzaid *et al.* (*36*) and extended later for analogous systems by Kanchuger and Byers (*37*). The σ^* for C_6H_5CO being 2.2 (*32*), the K_h in PGO hydration is calculated as 8.5×10^9 M^{-1}. Further, using the correlation **2,** suggested by Williams (*38*) for the pK_a of formaldehyde-hydrate, the pK_a of PGO–hydrate is calculated as ~11.2.

$$\log K_h = 1.68\sigma - 0.03 \quad [1]$$

$$pK_a = 14.4\sigma - 1.42 \quad [2]$$

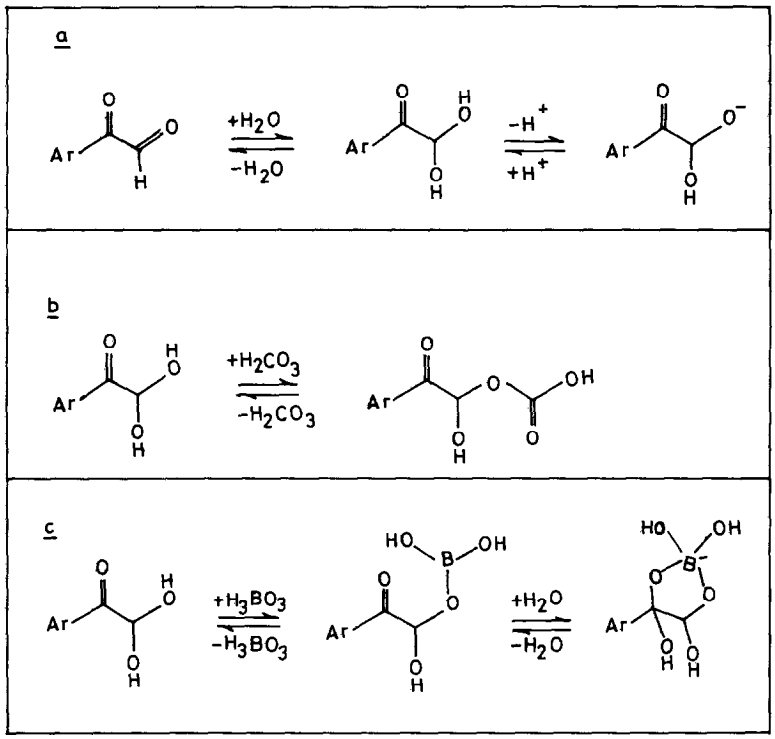
PGO is thus expected to be almost completely hydrated in water and to begin ionizing at pH >9.0. Being insulated from the aromatic ring, the aldehydic group in the molecule will experience only marginal substituent effects in its hydration or ionization equilibria. The large magnitude of K_h implies further that the equilibrium concentration of the aldehyde–hydrates will vary little irrespective of the substituent effects.

The buffer effects. The strongly nucleophilic primary amine buffers, such as Tris, are known to react with the α-dicarbonyl reagents and are unsuitable for the experiments involving the arginine modifications. A number of other buffers may be used (Table 1) and of these, as elaborated later, borate and bicarbonate have been of special interest. These buffers are also known to be unique in undergoing the acidic ionizations predominantly via the addition of OH^- and H_2O, rather than the loss of H^+, and in similarly reacting with other nucleophiles as well (*39*). The complexation of borate and bicarbonate with the arylglyoxal–hydrates, or their anions, is therefore to be expected, and appears in fact to occur according to the partial equilibria shown in Schemes IIb and IIc. Our results provide the direct evidence for the equilibria in borate (Fig. 1), while those in bicarbonate, being the well recognized property of aldehyde–hydrates (*39*), are inferred here from their kinetic consequences.

Bicarbonate was reported by Cheung and Fonda (*40*) to improve the reactivity of PGO, and was recommended as the buffer of choice in its use as the arginine modifier. Our results confirm that PGO, as well as DMAPGO, indeed are more arginine reactive in bicarbonate than in a number of other buffers (Table 1). The nature of concentration dependent effect of the buffer (Fig. 5) further suggests the action of bicarbonate as a nucleophilic catalyst in the reaction. Its effect is thus explainable on the assumption that the bicarbonate complexes (Scheme IIb) are about two times as reactive as the arylglyoxal–hydrates, and that the rate acceleration in increasing bicarbonate arises from the shift of equilibrium from the glyoxal–hydrates to the bicarbonate complexes. The enhanced reactivity of the bicarbonate complexes is in fact in conformity with the greater electron-withdrawing effect of –OCOOH than that of –OH, and in agreement with the nearly twofold difference in the Taft σ^* for $-CH_2OCOCH_3$ and $-CH_2OH$ (*34*).

Borate has been found to exercise a profound effect on the reactivities of the arylglyoxals. The effect stems from the complexation of the buffer with the glyoxal–hydrates (Scheme IIc) and the consequent action of boron as an intramolecular Lewis acid catalyst. While this activates the glyoxals toward the guanidine attack, it also promotes the partial hydration and consequent inactivation of their ketonic groups. The reactivity of a glyoxal, being sensitive to borate concentration as well as the substituent electronic effects, is thus determined by the position of equilibrium between the glyoxal–hydrates, their reactive borate complexes and the inert borate complexes (Scheme IIc). The activation of DMAPGO in increasing borate (Fig. 5) is thus attributable to the higher concentration of the reactive borate complexes, while the inactivation of PGO is attributable to the higher concentration of the inert borate complexes.

Borate was initially found, by Riordan (*21*), to enhance the reactivity of 2,3-butanedione. The buffer has consequently been used, by many workers, with other arginine modifiers as well (*5, 41–43*). The reagent activation and the restriction of the arginine–α-dicarbonyl reaction stoichiometry to 1 : 1 have been the perceived advantages arising from the use of borate. Our findings indicate, on the other hand, that borate is an inappropriate buffer for use with PGO as its overall effect is that of an inhibitor of this reagent. Only with electron-rich glyoxals, such as HOPGO



SCHEME II

and DMAPGO, has the catalytic effect of borate been found to operate, making it the buffer of choice in their use.

The remaining buffers investigated here (Table 1) have neither shown any noticeable catalytic nor inhibitory effects on the reactivities of the arylglyoxals. The reactivities of PGO as well as DMAPGO have been found to be insensitive to the concentration of bicine. The sharp initial decrease in the PGO reactivity noticed in Fig. 5C can be explained on account of the buffer substitution, with bicine displacing bicarbonate to nullify its favorable effect on the reactivity of the reagent.

Substituent effects. The Hammett correlations in bicarbonate and bicine buffers (Fig. 4) appear to reflect the typical substituent effects on the PGO reactivity. Indeed in all the buffers, except borate, PGO has been found to possess greater reactivity than DMAPGO (Table 1). The positive Hammett ρ, operative in the absence of borate, reflects the favorable effect of the electron-withdrawing substituents on the electrophilic reactivity of PGO. The exceptional reversal of this effect in borate has been found to stem from the substituent modulation of the buffer-mediated activation or inactivation of the glyoxals, and not due to any fundamental change in the reaction mechanism in this buffer.

The departure of HOPGO from the linear Hammett correlation in bicarbonate

and bicine buffers is an intriguing observation for which we are unable to provide a reasonable explanation at present.

Mechanistic implications. The magnitude of the Hammett ρ values and the nature of the buffer effects observed seem to imply that the rate-determining step in the arginine reaction with arylglyoxals consists in the nucleophilic attack at ketone, rather than aldehyde. The attack at aldehyde could constitute the first step in the reaction and facilitate the attack at ketone, making it the kinetically favorable intramolecular process. Such a mechanistic scheme can readily account for the selectivity of the α-dicarbonyls toward the bidentate nucleophiles, and explain their inertness toward $-NH_2$ and $-SH$, the functional groups in the lysine and cysteine side chains. Thus, even if these amino acids do react at the aldehyde center in PGO, their subsequent attack at the ketone will remain the entropically unfavorable intermolecular process. It is important to emphasize, however, that while free cysteine and lysine do show extremely poor reactivities toward PGO (*40*), there have been some reports implicating these aminoacids in the enzyme inactivations by the α-dicarbonyls (*10, 42, 44*). It is plausible that these exceptional situations arise accidentally, when the nucleophilic attack by the cysteine or lysine side chain, at the ketonic center in PGO, becomes the intramolecular process within the enzyme–inhibitor adsorptive complex.

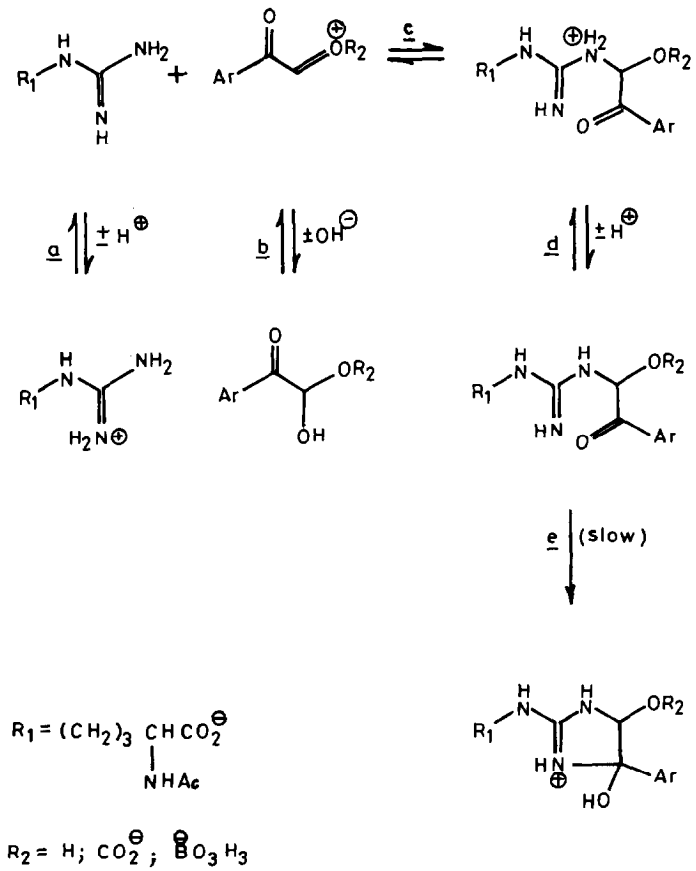
Under the pH conditions generally used for enzyme inactivations (pH 6 to 10), the reactivity of free arginine toward PGO has been reported to be proportional to the concentration of the unprotonated guanidine (*12*). Accordingly, a predissociative mechanism, involving proton transfer from guanidinium ion to OH^- prior to the rate-determining step, has been proposed in the arginine–PGO reaction. The independence from bicine concentration of the k_{obs} in the *N*-Ac-Arg reaction with PGO and DMAPGO (Fig. 5C) is in agreement with the proposed specific base catalysis in the reaction.

In rationalizing the chemistry in the arginine–PGO reaction, we would like to propose the mechanism illustrated in Scheme III. The mechanism envisages; (a) the pH-dependent deprotonation of guanidine determining the effective pool of the principal nucleophile; (b) the reversible hydration of arylglyoxals determining the dynamic pool of the effective electrophile; (c) the reversible addition of guanidine at aldehyde to furnish the monocarbinolamine intermediate; (d) the pH-dependent deprotonation of the intermediate; and (e) the C–N bond formation within the unprotonated monocarbinolamine as the rate-determining step in the overall reaction.

The proposed mechanism, which may be represented by the more familiar expression **3**, carries several kinetic implications that are verifiable.

$$A + B \overset{K}{\rightleftharpoons} A{\cdot}B \overset{k}{\rightarrow} A{\cdot}B \qquad [3]$$

The involvement of the preequilibrium step implies that the reaction will undergo a rate saturation when the concentration of either of the reactants is increased to a relatively large level. The rate saturation due to the large increase in *N*-Ac-Arg concentration has indeed been observed by us in a model reaction (*45*). The rate



SCHEME III

saturation due to the large excess of the reagent is relevant to the experimental situations involving the enzyme inactivations. A number of instances of rate saturation in enzyme inactivations by the α-dicarbonyls have indeed been observed (*5, 10, 46*). These have been attributed, as is customary, to an "affinity labeling" mechanism, implying the mediation of an adsorptive enzyme–reagent complex prior to the chemical bond formation. In light of the mechanism proposed here, it is equally plausible that such rate saturations are the result of the chemical equilibrium in the monocarbinolamine formation, rather than the noncovalent reagent–enzyme complexations. Further, according to the proposed mechanism the pH dependence of arginine modification is more likely to arise from the ionization of the monocarbinolamine intermediate, than that of arginine itself. The carboxypeptidase A inactivation by butanedione (*21*) has been observed to titrate to a lower pK_{app} than would be expected of the arginine titration. The identity of the concerned group is unknown, but it may well be the monocarbinolamine intermediate which, given its electron-withdrawing substituent, is expected to titrate to a pK_a lower than that of arginine.

The proposed mechanism provides a better focus in understanding the environmental influences that manifest in the arginine reactivities in proteins. The knowledge regarding the substituent and buffer effects is also relevant in the design and application of α-dicarbonyls as the arginine directed affinity labels (*47*), cross-linking reagents (*48*), or spectroscopic reporters.

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