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THE EFFECT OF ASSOCIATION WITH GUANIDINIUM IONS ON THE REACTIVITY OF A CARBOXYLATE OXYANION.

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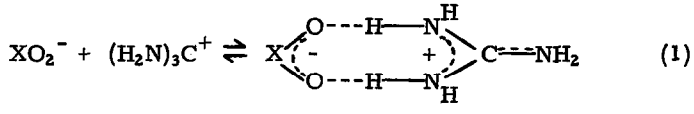
Our interest in electrophilic catalysis of acyl transfer reactions[1] has led us to investigate the non-bonded interaction between the guanidinium cation of enzymic arginine residues and carboxylate oxyanions. Crystallographic results for carboxypeptidase[2] indicate that a guanidinium-carboxylate interaction between arg-145 and the terminal carboxyl of the peptide chain appears important in binding and orienting the substrate. In lactate dehydrogenase, several arginines appear to function in catalysis and in binding of coenzyme and substrate; one arginine appears to be associated with the hydroxyl of lactate and to facilitate the proton removal that promotes hydride transfer and one is associated with the carboxylate anion of lactate.[3] It is of fundamental importance to determine both the extent of association between guanidinium cations and oxyanions (eq. 1) and the effect of that association on the reactivity of the oxyanion.



(1)

In this paper, we report the effect of association with guanidinium ion on the rate of reaction of a carboxylate anion by investigation of the monophenyl ester of succinic acid which hydrolyzes by a pH independent pathway between pH 4.5 and 9 involving intramolecular nucleophilic attack of the succinate carboxylate anion (eq. 2).[4] This system represents an uncomplicated case of nucleophilic catalysis by a carboxylate anion. Since an important driving force for the reaction should be the ion-dipole attraction between the carboxylate anion and the carbonyl dipole, we expected that association with guanidinium ion would inhibit the reaction. Rates of release of phenol from phenyl hydrogen succinate[5] were measured spectrophotometrically[4,6] at 30°C; pH was maintained at 7.00 in aqueous solution using a 0.1M sodium phosphate buffer. The rate constants for hydrolysis of phenyl succinate ($\underline{2}$) demonstrate inhibition by guanidinium ion (Table 1). The results in water indicate a negligible salt effect.

Table 1

The Effect of Guanidinium Ion of the Rate of Cleavage of 2

Solvent	$[C(NH_2)_3^+]$ (M.)	Ionic Strength (added NaCl)(M.)	$k_o(min^{-1})$ at 30.0°
H_2O	0	5.1	0.145
	0	5.1	0.139
	0.5	5.1	0.115
	1.0	5.1	0.110
	1.5	5.1	0.103
	2.0	5.1	0.0941
	3.0	5.1	0.0865
	4.0	5.1	0.0807
	4.5	5.1	0.0819
	4.5	5.1	0.0825
	5.0	5.1	0.0739
	0	1.1	0.142
	0	4.1	0.143
	0	0.1	0.143
	1.0	1.1	0.109
	2.0	2.1	0.0983
	3.0	3.1	0.0859
	4.0	4.1	0.0778
12 M CH_3OH in H_2O	0.0	0	0.0854
	0.5	0.5	0.0746
	0.75	0.75	0.0702
	1.0	1.0	0.0657
	1.25	1.25	0.0631
	1.5	1.5	0.0637
	1.75	1.75	0.0599
	2.0	2.0	0.0594
12 M CH_3CN in H_2O	0		0.106
	0.25	0.25	0.0922
	0.375	0.375	0.0899
	0.50	0.50	0.0877
	0.625	0.625	0.0855

Table 2

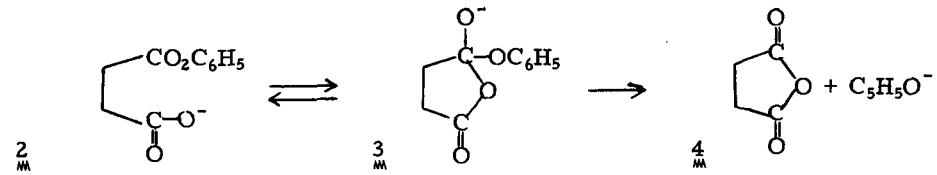
Association Constants and Rate Constants from Data in Table 1 (see Eqs. 3-7)

Solvent	$k_1(min^{-1})$	$k_2(min^{-1})$	$K(M^{-1})$
H_2O	0.143	0.053	0.55
12 M CH_3OH in H_2O	0.085	0.040	0.68
12 M CH_3CN in H_2O	0.106	0.072	3.3

Table 3

Rate of Cleavage of 2 in Water in the Presence of Arginine

Arginine(M)	Ionic Strength(M)	$k_o(min^{-1})$ at 30.0°
0.2	0.3	0.134
0.3	0.4	0.136
0.5	0.6	0.129
0.7	0.8	0.123
0.8	0.9	0.121
1.0	1.1	0.118



2 3 4 (2)

We interpreted the data on the basis of the following model:[7]

$$d[ArOH]/dt = k_1[S^-] + k_2[SGH] = k_o[S] \quad (3)$$

$$[SGH] = K[S^-][GH^+] \quad (4)$$

$$[S] = [SGH] + [S^-] \quad (5)$$

$$k_o = (k_1 + k_2K[GH^+])/(1 + K[GH^+]) \quad (6)$$

Rearranging eq. 6 gives:

$$k_o = \frac{1}{K} \cdot \frac{(k_1 - k_o)}{[GH^+]} + k_2 \quad (7)$$

Using eq. 7, k_o was plotted against $1/[GH^+]$ yielding k_2 from the intercept and K from the slope. The results are in Table 2.

Experiments with arginine also show inhibition (Table 3) but the solubility of arginine is not high enough to acquire data which would enable evaluation of k_2 through the use of eq. 7. However, if we make the approximation that $(k_1 - k_2)$ is roughly equal to k_1, eq. 8 results. When eq. 8

$$1/k_o = 1/k_1 + (K/k_1)[GH^+] \quad (8)$$

is applied to the data in Table 3, an approximate association constant can be estimated, $K = 0.20 M^{-1}$.

In other research, we utilized the effect of association (eq. 1) on acidity constants for measurement of association constants between guanidinium ions and oxyanions (eq. 1);[8] typical values (anion, $K_{association}$ in M^{-1}) are: $CH_3CO_2^-$, 0.37; $ClCH_2CO_2^-$, 0.43; HCO_3^-, 0.46; $H_2PO_4^-$, 1.4. The two association constants, $K = 0.20 M^{-1}$ for arginine and $0.55 M^{-1}$ for guanidinium ion, determined in the kinetic study reported here are comparable, therefore, to the association constants determined by pK measurements for carboxylate anions.

The inhibition in rate (k_1 roughly twice k_2) by association to guanidinium ion may not be due to the decrease in nucleophilicity of a carboxylate when hydrogen bonded to a guanidinium ion. Gaetjens and Morawetz[4] found that in the cleavage of aryl succinates the substituent effects in the aryl group are very large and indicate that resonance effects develop in the transition state based on p–NO_2 and p–C(O)CH_3 substituents. This suggests that the rate-determining step is a breakdown of the tetrahedral intermediate, 3, rather than the unlikely conclusion in the paper by Gaetjens and Morawetz that this is an S_N2 displacement at acyl carbon.[9] Therefore, the reason for inhibition by guanidinium ion probably involves shift of the equilibrium, 2⇄3, towards 2 caused by stronger complexing of GH^+ with 2 than with 3.

It is also possible that GH^+ both inhibits this reaction by complexing with the carboxylate ion in 2 and catalyzes the reaction by any one of several hypothetical means such as association of GH^+ with 3. Although this would lead to another association constant, K', and another rate

constant, k_3, since 2 is in equilibrium with 3, k_2[2-GH] is kinetically indistinguishable from k_3[3-GH]; therefore, a kinetic treatment based on reaction through any other complex such as [3-GH] is indistinguishable from the treatment leading to eq. 7 and K and k_2 (Table 2) represent all effects and interactions with 2 and 3. However, it seems reasonable that complexing of GH^+ with the carboxylate group of 2 is the strongest interaction in this system, so we conclude that these results give an approximate insight into the strength of association between guanidinium and carboxylate ions and the effect of such association on the reactivity of carboxylate anions.

In the following communication,[10] a catalytic effect of guanidinium cation on reaction at a phosphate anion is reported; tetramethylammonium ion is not effective in catalysis. Therefore, guanidinium ion has been demonstrated to have interactions with consequences for rates both for phosphates and carboxylates, and sodium ion and tetramethylammonium ion do not have those rate effects. However, the association constants are small and it is not clear that the associated structure shown in eq. 1 is predominant over all other structural interactions such as those involving one hydrogen bond or those occurring through the intermediacy of water molecules.

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REFERENCES AND NOTES

1. P. Haake and G. Hurst, J. Amer. Chem. Soc., 88, 2544 (1966); P. Haake, R. D. Cook, and G. Hurst, ibid., 89, 2650 (1967); P. Haake and P. S. Ossip, ibid., 93, 6919 (1971); P. Haake, G. Wallerberg, and J. Boger, J. Am. Chem. Soc., 93, 4938 (1971); D. A. Tyssee, L. P. Bausher, and P. Haake, ibid., 95, 8066 (1973); T. Koizumi and P. Haake, ibid., 95, 8073 (1973).

2. W. N. Lipscomb, Chem. Soc. Rev., 1, 319 (1972).

3. M. J. Adams, M. Buehner, K. Chandrasekhar, G. C. Ford, M. L. Hackert, A. Liljas, M. G. Rossman, I. E. Smiley, W. S. Allison, J. Everse, N. D. Kaplan, and S. S. Taylor, Proc. Nat. Acad. Sci. U. S., 70, 1968 (1973).

4. E. Gaetjens and H. Morawetz, J. Amer. Chem. Soc., 82, 5329 (1960).

5. Prepared by method of C. A. Bischoff and A. Vonhendenstron, Chem. Ber., 35, 4076 (1902).

6. The rate constants were determined by a least squares treatment of $\ln(A_{inf}-A)$ vs time. The infinity point was chosen to be that value (to three significant figures) in the local region of the experimental infinity point which yielded a minimum slope variance. All infinity points chosen in this manner varied less than 3% from the experimental point.

7. Abbreviations: S^- = 2, SGH = (2 complexed with GH^+), GH^+ = guanidinium ion, k_o = observed rate constants in Table 1, k_1 = rate constant for reaction of S^-, k_2 = rate constant for reaction of SGH.

8. B. Springs and P. Haake, Bioorganic Chemistry, in press.

9. Principles which are applicable here and apply to the relative rates of formation and breakdown of intermediates are discussed in R. D. Cook, C. E. Diebert, W. Schwarz, P. C. Turley, and P. Haake, J. Amer. Chem. Soc., 95, 8088 (1973).

10. B. Springs and P. Haake, Tetrahedron Lett., 3223 (1977).