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CATALYSIS BY GUANIDINIUM IONS IN NUCLEOPHILIC ATTACK AT A PHOSPHATE MONOANION. A MODEL FOR A CATALYTIC FUNCTION OF ARGININE AT ENZYMIC ACTIVE SITES.

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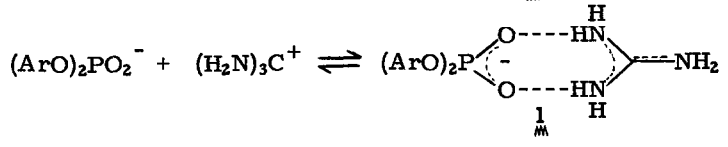
Our interest in the fundamental chemistry of phosphates[1] led us to investigate the effect of guanidinium cation on the reactivity of a phosphate anion and we report the results in this communication. In the previous communication, we evaluated the effect of complexing to guanidinium cation on the rate of reaction of a carboxylate anion; both rate and equilibrium constants for the associated species were determined.[2] This research has fundamental importance for understanding enzymes in which arginine residues are important in interactions with substrates and coenzymes; arg-35 and arg-87 in staphyloccal nuclease appear to facilitate nucleophilic attack at the phosphate anion of nucleic acids.[3]

We have investigated the effect of association to guanidinium cations (eq. 1) on the rate of attack of anions on a diaryl phosphate anion (1). Bis-(p-nitrophenyl) phosphate was crystallized



from acetonitrile and ether as the cyclohexylammonium salt, mp 186-7°.[4] Pseudo first order rate constants were determined spectrophotometrically (Tables 1, 2, 3).[5] Second order rate constants (k_2) for hydroxide and fluoride attack on bis-(p-nitrophenyl) phosphate were determined from the slope of the pseudo first order rate constant, k_{obs}, vs [OH] or [F^-] (Table 1). In the attack of hydroxide on phosphate monoanion (1), we observed catalysis by addition of guanidinium ion (GH^+) (Table 3) but this could be due to either nucleophilic attack by guanidine (G) which is a strong base or to guanidinium (GH^+) catalysis of attack by hydroxide. These are kinetically equivalent (eqs. 2, 3) and could not be distinguished.

TABLE 1

Second-order rate constants for OH^- and F^- attack on bis-(p-nitrophenyl) phosphate in aqueous solution containing 4.0 M. guanidinium chloride.

Nucleophile (M)	$k_{obs}(sec^{-1})$	$k_2(M^{-1}sec^{-1})$
$0.4F^-$	14.6×10^{-7}	
$0.2F^-$	6.75×10^{-7}	3.50×10^{-6}
$0.1F^-$	3.75×10^{-7}	
$0.6OH^-$	13.8×10^{-6}	
$0.4OH^-$	8.43×10^{-6}	2.85×10^{-5}
$0.2OH^-$	2.31×10^{-6}	

TABLE 2

The effects of guanidinium ion on second-order rate constants for attack of hydroxide ion on bis-(p-nitrophenyl) phosphate

$[(H_2N)_3C^+]$	$[(H_2N)_2CNH]$	$[HO^-]$	μ	(sec^{-1}) 10^5k	$(M^{-2}sec^{-1})$ $10^4k/[HO^-][GH^+]$
2.0	0.20	0.039	5.2	1.29	16.3
2.0	0.50	0.097	2.5	3.08	15.9
2.0	0.50	0.097	2.5	2.79	14.4
2.0	0.50	0.097	3.5	3.09	15.9
2.0	0.50	0.097	4.5	3.03	15.6
5.0	0.50	0.039	5.5	2.87	14.3
5.0	0.75	0.059	5.75	3.15	10.7
5.0	1.0	0.078	6.0	7.35	18.8
5.0	0.50	0.039	5.5	3.48	17.8

TABLE 3

Guanidinium ion effect on the reaction of 0.4 M. fluoride ion with bis-(p-nitrophenyl) phosphate in aqueous solution[a]

GH^+ (M)	$10^6k_{obs}(sec^{-1})$	$10^6k_{calc}(sec^{-1})$[b]
0	0.140	0.140 (from k_2)
0.25	0.285	0.30
0.50	0.428	0.44
0.75	0.544	0.58
1.0	0.700	0.70
1.5	0.931	0.92
2.0	1.13	1.11
2.5	1.27	1.28
3.0	1.44	1.42
4.0	1.46	1.67

a All reactions were brought to a total constant ionic strength of 4.4 M with tetramethylammonium chloride including guanidinium chloride and 0.4 M NaF.

b Calculated from $K = 0.18\ M^{-1}$, $k_2 = 0.35 \times 10^{-6} sec^{-1}$, $k_3 = 9.5 \times 10^{-6} sec^{-1}$.

$$K_a = [G][H^+]/[GH^+] = [G]K_w/[GH^+][HO^-] \quad (2)$$

$$k_2^+[HO^-][GH^+][\text{phosphate}] = (k_2^+ K_w/K_a)[G][\text{phosphate}] \quad (3)$$

In the attack of fluoride anion on the phosphate anion, the reaction is first order in fluoride, $k_2 = 3.50 \times 10^{-6} M^{-1}sec^{-1}$ which is approximately one-tenth the value of k_2 for hydroxide, $2.85 \times 10^{-5} M^{-1}sec^{-1}$(Table 1). The spontaneous hydrolysis of bis-(p-nitrophenyl) phosphate has been reported as $6.30 \times 10^{-8} sec^{-1}$.[4] Table 3 displays pronounced catalysis by guanidinium ion, GH^+. Using the kinetic model in eqs. (4)-(9), we deduce the following results: a)From the intercept

$$v = k_{obs}[P_t] \quad (4)$$

$$k_{obs} = k_2[F^-][P^-] + k_3[F^-][GH^+ \cdot P^-] \quad (5)$$

$$[GH^+ \cdot P^-] = [GH^+][P^-]K \quad (6)$$

$$[P_t] = [GH^+ \cdot P^-] + [P_t] \quad (7)$$

$$k_{obs}/[F^-] = (k_2 + k_3 K[GH^+])/(1 + K[GH^+]) \quad (8)$$

$$k_{obs}/[F^-] = k' = k_3 - \frac{1}{K} \cdot \frac{(k' - k_2)}{[GH^+]} \quad (9)$$

of $k' = k_{obs}[F^-]$ plotted vs. $[GH^+]$ (eq. 5) we obtain $k_2 = 0.35 \times 10^{-6} M^{-1}sec^{-1}$. b) From a plot of k' vs. $(k'-k_2)/[GH^+]$ we obtain k_3 as the intercept and $-1/K$ as slope giving $k_3 = 9.5 \times 10^{-6} M^{-1}$ sec^{-1} and $K = 0.18\ M^{-1}$. Using these values and eq. 8 we have calculated k values for comparison with the experimental values (Table 3); the agreement appears to be satisfactory.

The values of k_2 and k_3 demonstrate that association to a guanidinium ion increases the rate of attack by F^- by a factor of 27. Although this must be predominantly an electrostatic effect, there may also be an induced change in structure of the phosphate which changes the nonelectrostatic barrier to approach of the nucleophile. Since $(CH_3)_4N^+Cl^-$ was used to maintain ionic strength it appears that the catalysis is a specific effect of guanidinium ion and not some general effect of large cations. In the previous communication, we demonstrated that Na^+ does not have the rate effects of guanidinium ion, $C(NH_2)_3^+$.

Electrostatic theory states[6] that the energy of interaction in species such as (1) will be given by eq. 10 where Q_1 and Q_2 are the charges on the ions, e is the electronic charge, D is the dielectric constant, and r is the distance

$$\Delta G(\text{electrostatic}) = -Q_1Q_2e^2/Dr \quad (10)$$

between charges. However, application of this apparently simple equation is complex due to the problems of estimating the distance and dielectric constant between charges and dipoles in the transition state or at an enzymic active site.

The results reported here should be useful in analysis of the mechanism of action of enzymes. The catalytic factor of 27 found in this study would be expected to be larger if the reaction were occurring at an enzymic active site with a dielectric constant lower than the dielectric constant of water.[7]

The association constant of $0.18\,M^{-1}$ which was found in this study is very small--much smaller than we found for $H_2PO_4^-$ in a study of association constants.[8] Since half association to the bis-aryl phosphate anion would require 5.7 M guanidinium ion and each guanidinium ion has 3 $-NH_2^{\delta+}$ groups, the concentration of $-NH_2^{\delta+}$ groups is approximately 17 M when the phosphate anion is half associated. Half association is the state where equal numbers of molecules are associated to guanidinium ion and to water. Therefore, in the case of this particular anion, the $-NH_2^{\delta+}$ groups of guanidinium chloride are only slightly more strongly associated to the phosphate anion than are water molecules. It is not clear that guanidinium ions always associate with oxyanions with the structure shown in 1 and other structures may have to be considered. Since formation of 1 causes a considerable loss of entropy, structures with single hydrogen bonds may be comparably stable to 1 in water. However, in an enzymic active site where the dielectric constant is lower and where there is less competition with water, one would expect 1 to be the predominant structure of guanidinium-oxyanion complexes; then, the two hydrogen bonds in 1 might be effective both in orienting a phosphate substrate and in catalyzing nucleophilic attack.

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