Kinetic Evidence for an Acylpyridinium Intermediate in Hydrolysis of *p*-Nitrophenyl Alkanoates Catalyzed by a Polyamide with 4-(Dialkylamino)pyridine Groups

Shanghao Liu and Wilmer K. Fife*

Department of Chemistry, Indiana University−Purdue University at Indianapolis, 402 N. Blackford Street, Indianapolis, Indiana 46202

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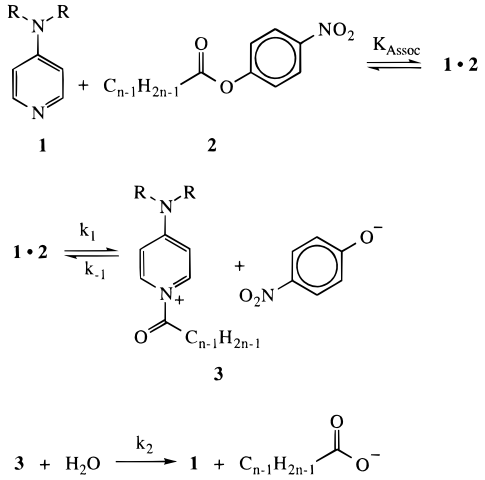
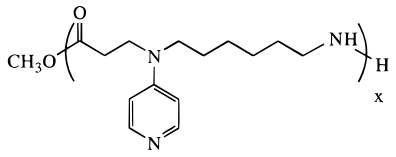
Pyridine derivatives such as 4-(dimethylamino)pyridine (DMAP) and its analogs are widely recognized as supernucleophilic, acyl-transfer catalysts in nonaqueous media.[1] Recently, a number of polymeric 4-(dialkylamino)pyridines (poly-DAAP) have been synthesized and evaluated as enzyme mimics in catalysis of hydrolysis of *p*-nitrophenyl alkanoates.[2−4] Data from these studies document the high activity of some poly-DAAP catalysts in aqueous media. One of the systems also shows enzyme-like substrate selectivity.[4] The mechanism for catalysis is usually assumed to involve attack by nucleophilic DAAP **1** at the carbonyl group of substrate **2** and formation of an N-acyl DAAP intermediate **3** (Scheme 1). Belief that intermediates such as **3** are key participants in the acyl-transfer reactions of DMAP and related compounds is based on their isolation from nonaqueous media of low nucleophilicity and characterization by UV spectrophotometry.[1,5] Although the intermediacy of **3** in reactions of acid chlorides and anhydrides with DAAP appears well established, questions remain about the mechanism of acyl-transfer reactions of phenyl esters such as **2** when catalyzed by DAAP. A serious impediment to definition of mechanism in these reactions is the inability to directly observe the presence of **3** at 310−315 nm due to strong absorption in that spectral region by **2** and its hydrolysis product, *p*-nitrophenoxide (Scheme 1). An alternative approach is available when formation of **3** is irreversible and fast compared to its rate of hydrolysis (i.e., $k_1 \gg k_{-1}$ and k_2, Scheme 1). For this circumstance, "burst" kinetics are observed as evidenced by a rapid increase in absorbance at 400 nm due to the release of an equivalent of *p*-nitrophenoxide that accompanies formation of **3**. Then, a much slower, turnover stage of the reaction ensues in which excess substrate is hydrolyzed via a sequence of slow hydrolysis of **3** followed by rapid acylation of free DAAP sites of **1** to restore the supply of **3**. Klotz and co-workers reported rapid formation and subsequent slow hydrolysis of an *N*-acetyl DAAP intermediate when a poly-DAAP was treated with acetic anhydride.[2b] Vaidya and Mathias investigated this possibility with their poly-DAAP variant, found no burst, and concluded that the acylation step to form an *N*-acyl DAAP intermediate **3** is rate-limiting.[3] Fife and co-workers also failed to observe a "burst" of *p*-nitrophenoxide in reactions of a different poly-DAAP and **2** in aqueous methanol.[4]

In this communication, we report the first observation of "burst" kinetics for DAAP-catalyzed hydrolysis of *p*-nitrophenyl esters. The catalyst is the novel polyamide **4** functionalized with DAAP groups integrated into the backbone of the polymer. The reaction was carried out in aqueous Tris buffer (0.05 M, pH 8.0) at 30 °C with four *p*-nitrophenyl alkanoates **2** (n = 6, 8, 10, 12). Figures 1 and 2 illustrate representative results

Scheme 1. Role of *N*-Acyl DAAP Intermediate 3 in DAAP Catalysis of Hydrolysis of *p*-Nitrophenyl Alkanoates 2



from the various experiments, and Table 1 summarizes kinetic data. Figure 1 shows time- and catalyst-concentration-dependent release of *p*-nitrophenoxide from **2** ($n = 10$). The results clearly indicate proportionality between magnitude of burst of *p*-nitrophenoxide and catalyst concentration. A control experiment with no catalyst gives no burst, and hydrolysis of **2** proceeds at a rate less than those of second stages of catalyzed reactions. The acylation step is first order in catalyst concentration and exhibits saturation at high substrate concentration for **2** ($n \geq 8$). These results are consistent with the Michaelis−Menten model for enzyme kinetics.[5,6] The inhibitory influence of added *p*-nitrophenoxide is displayed in the data represented with filled circles (Figure 1). The presence of 2×10^{-5} M *p*-nitrophenoxide causes decreases in the magnitude of the burst and in rates of acylation of DAAP residues (k_1 step) and hydrolysis of *N*-acyl intermediate (k_2 step).



The kinetic parameters for these reactions are listed in Table 1. Bursts of *p*-nitrophenoxide larger than calculated concentrations of free DAAP sites are believed to result from rapid deprotonation of a fraction of pyridinium sites during acylation of the original free DAAP sites. This is particularly true for reaction mixtures that contain excess substrate. Unlike enzymes, the DAAP groups of catalyst **4** exhibit variable pK_a values that decrease with increased fraction of protonated and/or acylated DAAP residues. Acid−base equilibria as well as distribution of catalyst, substrate, and key intermediates among several kinds of aggregates and the bulk solution are dependent on the concentrations of these species. Note that agreement between magnitude of *p*-nitrophenoxide burst (4.3×10^{-6} M) and concentration of unprotonated DAAP sites on catalyst **4** at pH 8.0 (4.0×10^{-6} M) is quite good in the experiments with equimolar quantities (2.0×10^{-5} M) of **2** ($n = 10$) and **4**. Furthermore, the values of k'_1 (0.8 ± 0.1 min^{-1}) and k_2 (0.075 ± 0.004 min^{-1}), the rate

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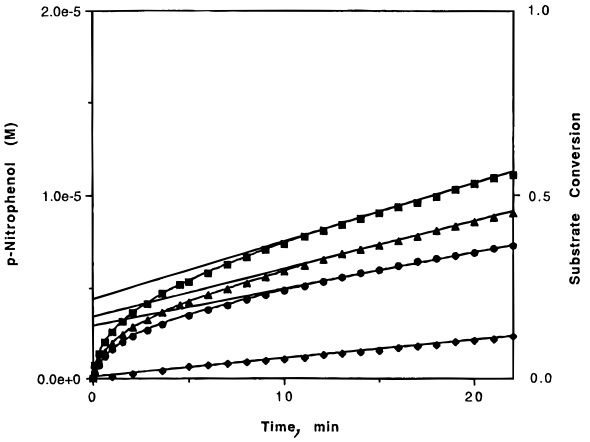


Figure 1. Time-dependent liberation of *p*-nitrophenoxide and extent of substrate conversion during hydrolysis of **2** ($n = 10$) in the absence and presence of **4** and *p*-nitrophenol in 0.05 M aqueous Tris buffer, pH 8.0, 30 °C, [**2**] = 2×10^{-5} M: (■) [**4**] = 2.0×10^{-5} M; (▲) [**4**] = 1.0×10^{-5} M; (●) [**4**] = 1.0×10^{-5} M, [*p*-nitrophenol] = 2.0×10^{-5} M; (◆) no catalyst and other materials.

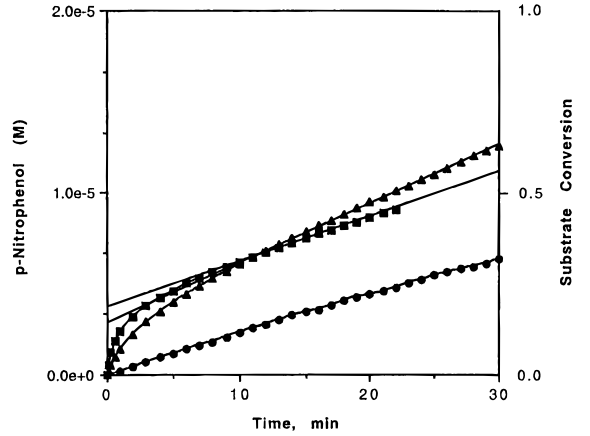


Figure 2. Time-dependent liberation of *p*-nitrophenoxide and extent of substrate conversion during hydrolysis of **2** in the presence of **4** in 0.05 M aqueous Tris buffer, pH 8.0, 30 °C, [**4**] = 1.0×10^{-5} M, [**2**] = 2.0×10^{-5} M: (■) **2** ($n = 12$); (▲) **2** ($n = 8$); (●), **2** ($n = 6$).

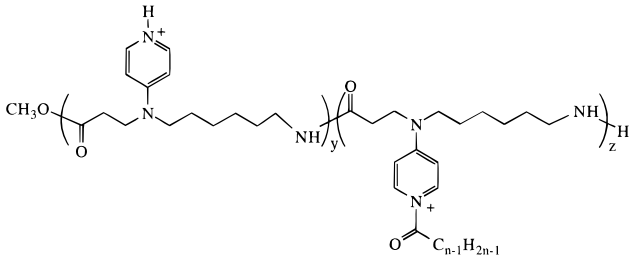
Table 1. Kinetic Parameters for Hydrolysis of 2 ($n = 10$) Catalyzed by Polyamide 4[a]

total cat. (M)	free DAAP[b] (M)	*p*-nitrophenoxide[c] (M)	k'_1[d] (min^{-1})	k_2[e] (min^{-1})
5.0×10^{-6}	1.0×10^{-6}	2.9×10^{-6}	0.94	0.079
1.0×10^{-5}	2.0×10^{-6}	3.3×10^{-6}	0.83	0.079
1.0×10^{-5} [f]	2.0×10^{-6} [f]	2.8×10^{-6} [f]	0.81[f]	0.071[f]
1.5×10^{-5}	3.0×10^{-6}	4.0×10^{-6}	0.81	0.072
2.0×10^{-5}	4.0×10^{-6}	4.3×10^{-6}	0.76	0.072

[a] Reaction conditions: [**2**] = 2×10^{-5} M, 0.05 M aqueous Tris buffer, pH 8.0, 30 °C. [b] Calculated from total concentration and $pK_a = 8.61$ of the catalyst **4**. [c] Intercept of linear extrapolation of slower kinetic phase in Figure 1. [d] Apparent first-order rate constants $k'_1 = k_1 K_{assoc}[\mathbf{2}]/1 + K_{assoc}[\mathbf{2}]$ for formation of the *N*-acylpyridinium intermediate **5** derived from **4** were obtained as slopes of plots of $\ln\{[PNP]_{burst}/([PNP]_{burst} - [PNP]_t)\}$ vs time *t*, where $[PNP]_{burst}$ and $[PNP]_t$ are the concentrations of *p*-nitrophenoxide formed during the fast acylation step (column 3 of the table) and at time *t*, respectively (ref 6, Chapter 3). [e] First-order rate constant for hydrolysis of the *N*-acylpyridinium intermediate (velocity of hydrolysis of **5** divided by $[PNP]_{burst}$). [f] In the presence of 2×10^{-5} M *p*-nitrophenol.

constants for acylation and deacylation of **4**, fit the mechanism proposed in Scheme 1.

Figure 2 shows the effect of alkyl chain length of **2** on the reaction. The most hydrophilic ester **2** ($n = 6$), gives no burst, but the two esters with longer alkanoate chains give typical burst kinetics where k_1 is proportional to *n*. However, k_2, the rate constant for hydrolysis of **3**, decreases with increasing *n*. The influence of changes in hydrophobicity of substrate on reaction kinetics also is consistent with the proposed scheme. Increases in rate of acylation of **4** are expected to accompany increases in hydrophobicity of **2** due to enhanced binding, which shifts the catalyst−substrate equilibrium in favor of complex formation, i.e., **4** + **2** ⇌ **4·2**. On the other hand, enhanced hydrophobicity of **5**, the *N*-acylpyridinium intermediate related to **4**, due to the increased chain length of the acyl group is expected to place **5** in a less polar region of the reaction system, effectively making it less accessible to water.



5, y + z = x

It is important to note that the effect of alkanoate chain length of *p*-nitrophenyl esters **2** on reactivity in the presence of **4** is quite different from similar solution reactions catalyzed by hydrophilic monomolecular bases. In this study, the reactivity of **2** in the presence of **4** is directly proportional to the chain length and hydrophobicity of **2**. Work from the laboratories of Menger,[10] Knowles,[11] Guthrie,[12] Jiang,[13] and others has clearly demonstrated that *p*-nitrophenyl esters with alkanoate chain lengths greater than six carbons aggregate in aqueous media at the concentrations used in this study. Furthermore, the reactivity of aggregates of **2** is considerably less than that of comparable solution species. Thus, the behavior of the **2·4** system more closely resembles the behavior of enzyme−substrate systems than simple solution processes. Continuing research currently underway in our laboratory is focused on the relationship between catalyst microstructure and expression of catalytic efficiency and specificity toward substrate.

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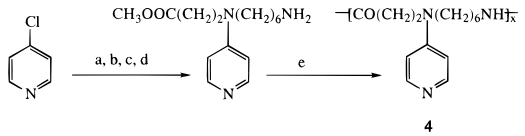
(8) The poly-DAAP **4** was synthesized by the reaction sequence below and characterized by ^{1}H NMR and IR.



4

a) $H_2N(CH_2)_6NH_2$, 3 days, 130 °C; b) CBZCl, Et_3N/CH_2Cl_2, 0 °C, 30 min; c) CH_2=CHCOOCH$_3$, reflux, 24 h; d) Pd-C/cyclohexene, CF_3COOH/MeOH, 4 h; e) 80-130 °C, Ar, 48 h.

Its pK_a as determined by spectrophotometric titration is 8.61 and its degree of polymerization (X) is greater than 20 as estimated from the ratio of integrated signals for protons in the backbone to those in terminal groups. Although **4** contains a primary amine function, its low concentration relative to DAAP groups and high degree of protonation rule out a significant contribution to catalysis. The details of synthesis of this and other similar poly(4-(dialkylamino)-pyridines) are the subject of another paper in preparation.

(9) Kinetic measurement: Reaction mixtures were made up in a 1.00-cm quartz cuvette. The cuvette was filled with 3.00 mL of Tris buffer (0.05 M, pH 8.0). A stock solution of catalyst **4** in methanol (usually 20 μL) was added by microsyringe, and in selected experiments, a certain amount of *p*-nitrophenol in acetonitrile was also introduced by microsyringe. The solution was then equilibrated for 10 min in the thermostated cell compartment (30 ± 0.1 °C) of a Perkin-Elmer Lambda 19 spectrophotometer. An appropriate aliquot (usually 20 μL) of a stock solution of **2** in acetonitrile was added by microsyringe. The reaction mixture was quickly mixed by shaking, and the absorbance at 400 nm was recorded as a function of time. Duplicate runs generally showed a measurement error of less than 6% for absorbance vs time data.

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