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Enhanced Methylation Rate within a Foldable Molecular Receptor

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Received August 25, 2004

A *N,N*-(dimethylamino)pyridine monomer is incorporated into the backbone of a *m*-phenyleneethynylene oligomer such that the pyridine nitrogen is located on the interior surface of the binding cavity in the folded structure of the oligomer. For an oligomer having a chain length of 13 monomer units, competitive inhibition experiments reveal that methyl iodide binds weakly within the oligomer cavity with an association constant $K_a = 2\ M^{-1}$, and the oligomer−methyl iodide complex reacts with unimolecular rate constant $k_u = 0.082\ s^{-1}$ to provide the methylated product. The effective molarity is calculated to be 230 M by comparison of k_u for the 13-mer with the second-order rate constant for a 3-mer that is too short to fold and thus unable to bind methyl iodide.

Introduction

Rate enhancements for "intramolecular" reactions in which two or more reactants are constrained in close proximity are ubiquitous in nature and have been demonstrated extensively in synthetic systems.[1] Menger has proposed that the rates of these reactions depend on the length of time that the reactants are held within a critical distance of one another,[1b] and the effective molarity (EM) parameter is used to quantify the level of "intramolecularity" in the reactions.[2] Structurally rigid receptors have been used extensively to increase the effective molarity of reactants. However, the size, shape, and functional groups that define the binding sites of these receptors are difficult to modify. Analogous to peptide chains, the modular construction of synthetic oligomers from a diverse monomer pool may provide considerable control over cavity shape and functionality. But just as polypeptides require a distinct conformation to function as enzymes, synthetic oligomers must fold into the desired receptor conformation, an event that is made challenging by the large ensemble of alternative possibilities.

Phenyleneethynylene (PE) oligomers previously studied by our group and others[3] have been found to adopt a compact helical conformation in polar solvents such as acetonitrile,[4] generating a solvophobic cavity that is capable of molecular recognition.[5,6] The surface of the binding cavity can be chemically modified without significantly disrupting the folded structure of the oligomer.[7] We have demonstrated that a *N,N*-(dimethylamino)-pyridine (DMAP) monomer can be incorporated into the backbone sequence of a PE oligomer, such that the pyridine nitrogen is located on the interior of the binding cavity.[7a] The folded conformation of the oligomer dramatically increased the methylation rate of the DMAP moiety, but the origin of this rate acceleration remained elusive.[8] The kinetic experiments described here show how the oligomer functions as a foldable molecular receptor to bind methyl iodide in the cavity interior, increasing the effective molarity and accelerating methylation of the DMAP moiety.

Results and Discussion

Oligomers **1** and **2** were previously synthesized as DMAP analogues. With use of UV spectroscopy, the methylation rates of **1** and **2** were measured in both acetonitrile and chloroform under pseudo-first-order reaction conditions with a large excess of methyl iodide (Scheme 1). The second-order rate constants (k_2) for the reactions reveal that in acetonitrile, oligomer **2** reacts over 400 times faster than oligomer **1**, with a difference in free energy of activation ($\Delta\Delta G^{\ddagger}$) of 3.6 kcal·mol^{-1}. However, in chloroform only a 2-fold rate enhancement

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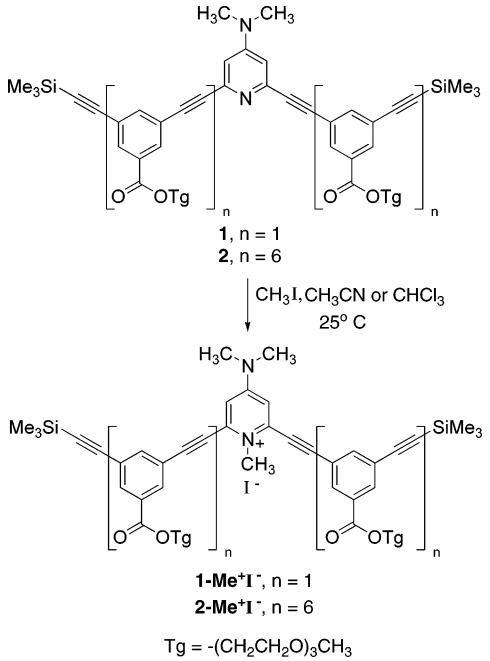
10.1021/jo048506d CCC: $27.50
Published on Web 11/10/2004

SCHEME 1. Methylation of Pyridine-Containing PE Oligomers



is observed, corresponding to a $\Delta\Delta G^{\ddagger}$ value of 0.4 kcal·mol^{-1}.[8] In chloroform, both **1** and **2** exist in random conformations, but in acetonitrile, **2** adopts a helical conformation,[7a] implicating oligomer folding as the key structural change contributing to the large rate enhancement.

We hypothesized that the increased reaction rate for the folded oligomer relative to its unfolded counterpart may be attributable to binding of methyl iodide (M) in the solvophobic interior cavity (C) of the helix. However, if the reaction mechanism involves binding of methyl iodide prior to nucleophilic attack by the pyridine nitrogen (eq 1), then second-order kinetics may no longer be sufficient for characterizing the reaction rate. Instead,

$$\mathrm{C} + \mathrm{M} \overset{K_a}{\rightleftharpoons} \mathrm{C{\cdot}M} \xrightarrow{K_u} \mathrm{P} \qquad (1)$$

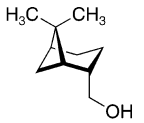
the observed rate constant (k_{obs}) would be consistent with eq 2,[9] in which K_a is the association constant for formation of a 1:1 complex of oligomer with methyl iodide and k_u is the unimolecular rate constant for conversion of the oligomer−methyl iodide complex to the methyl pyridinium product. According to eq 2, a plot of k_{obs} vs [M]

$$k_{obs} = \frac{k_u K_a[\mathrm{M}]}{K_a[\mathrm{M}] + 1} \qquad (2)$$

should be linear at low concentrations of methyl iodide, but reach a plateau at higher concentrations as the host becomes saturated with guest.

Methylation of oligomer **2** was carried out with varying concentrations of methyl iodide to determine the level of correlation between the observed rate constant and the trend predicted by eq 2. The relationship between k_{obs} and [M] was found to be approximately linear, justifying the earlier treatment of the reaction rates using second-order kinetics, but failing to establish the reaction mechanism. One possibility is that methyl iodide does not bind in the helical cavity prior to the reaction. However, the data do not exclude the possibilities that either the value of K_a is very low or the oligomer is capable of binding multiple methyl iodide molecules. In both of these cases, high concentrations of methyl iodide would be required to reach saturation, accounting for the linear relationship of k_{obs} to [M] over the concentration range studied.

Experimental limitations prohibit measuring the reaction rate at methyl iodide concentrations greater than 0.2 M.[10] Thus, an alternate method was required to establish the reaction mechanism. We hypothesized that an inhibitor capable of occupying the helical cavity of the oligomer and blocking the pyridine nitrogen might obstruct binding of methyl iodide, resulting in a decrease in reaction rate if methyl iodide binding is in fact a crucial step in the reaction mechanism. Monoterpenes have been shown to bind in the helical cavity of PE oligomers,[5a] and hydrogen bonding interactions have been used to enhance binding between hydroxyl-functionalized guests and pyridine-containing PE oligomers.[6,11] On the basis of these criteria, (−)-myrtanol appeared to be a suitable inhibitor for oligomer **2**.



(1*S*,2*S*,5*S*)-(-)-myrtanol

The kinetic model for competitive inhibition is shown in eq 3,[12] where K_i is the association constant for formation of a 1:1 complex of oligomer and inhibitor (In).

$$\begin{gathered} \mathrm{C} + \mathrm{M} \overset{K_a}{\rightleftharpoons} \mathrm{C{\cdot}M} \xrightarrow{K_u} \mathrm{P} \\ \mathrm{C} + \mathrm{In} \overset{K_i}{\rightleftharpoons} \mathrm{C{\cdot}In} \end{gathered} \qquad (3)$$

In light of the preceding discussion, it is also necessary to consider a kinetic model in which multiple methyl iodide molecules bind in the cavity prior to nucleophilic attack. Additionally, the ability of the oligomer−inhibitor complex to react with methyl iodide, though presumably at a much slower rate than with the free oligomer, must be considered.

The methylation rate of **2** was measured in the presence of varying concentrations of (−)-myrtanol, and the observed rate constant was found to decrease in response to increases in (−)-myrtanol concentration (Figure 1a). In a control experiment, the methylation rate of **1** was measured over the same concentration range of (−)-myrtanol, with no observed inhibitory behavior (Figure 1b). Nonlinear least-squares curve fitting was used to

(9) For an example, see: Banerji, K. K. *J. Org. Chem.* **1988**, *53*, 2154−2159.

(10) When present in high concentrations (>1% v/v), methyl iodide may destabilize the folded conformation of the oligomer and alter the polarity of the solvent, giving rise to significant errors in the measured rate constant.

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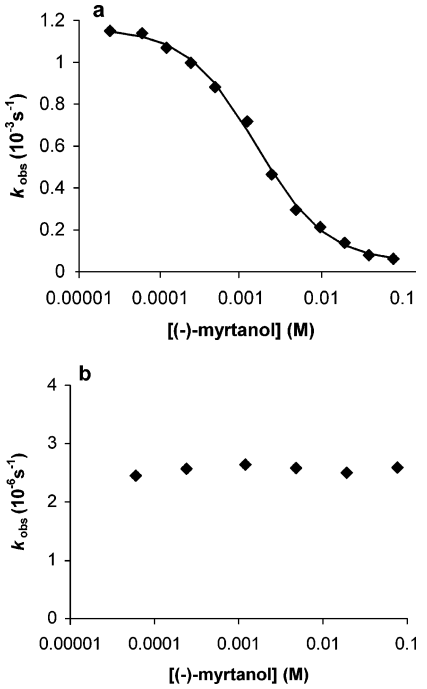

FIGURE 1. (a) Inhibition of **2** by (−)-myrtanol ([**2**] = 4.78 μM, [M] = 6.88 mM, CH_3CN, 25 °C). The solid line represents the nonlinear least-squares curve fit. (b) Methylation of **1** in the presence of (−)-myrtanol ([**1**] = 20.0 μM, [M] = 6.88 mM, CH_3CN, 25 °C).

TABLE 1. Parameters from Curve Fitting of the Inhibition of 2 by (−)-Myrtanol[a,b]

Mechanism A:

$$C + M \underset{}{\overset{K_a}{\rightleftharpoons}} C{\cdot}M \xrightarrow{k_u} P$$

$$C + In \underset{}{\overset{K_i}{\rightleftharpoons}} C{\cdot}In \xrightarrow[M]{k_{in}} P$$

Mechanism B:

$$C + M \underset{}{\overset{K_a}{\rightleftharpoons}} C{\cdot}M \xrightarrow{k_u} P$$

$$C{\cdot}M + M \underset{}{\overset{K_{a2}}{\rightleftharpoons}} C{\cdot}M_2 \xrightarrow{k_{u2}} P + M$$

$$C + In \underset{}{\overset{K_i}{\rightleftharpoons}} C{\cdot}In \xrightarrow[M]{k_{in}} P$$

mechanism	k_{in} (M^{-1} s^{-1})	K_i (M^{-1})	K_a (M^{-1})	K_{a2} (M^{-1})	k_u (s^{-1})	k_{u2} (s^{-1})
A	0.0062	660	2.1		0.082	
B	0.0062	720	13	33	0.059	0.036

[a] [**2**] = 4.78 μM, [M] = 6.88 mM, CH_3CN, 25 °C. [b] See Supporting Information for error values associated with each parameter.

compare the kinetic data from the methylation of **2** to a variety of mechanistic models. A suitable fit was obtained for mechanism A, in which the reaction proceeds primarily through a 1:1 complex of oligomer with methyl iodide, but with the oligomer−inhibitor complex also being marginally reactive toward methyl iodide (Table 1). The data also fit well with mechanism B, in which a second molecule of methyl iodide binds in the cavity prior to the reaction, so we reexamined the data from the initial binding study in hopes that it might distinguish between the two mechanisms. Figure 2 compares the observed

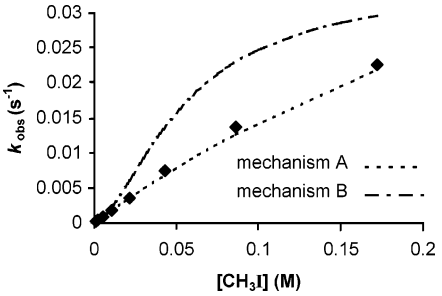

FIGURE 2. Comparison of k_{obs} vs [M] determined experimentally (◆) and calculated by using parameters from Table 1.

rate constants at varying concentrations of methyl iodide with the rate constants predicted by using the values of K_a, K_{a2}, k_u, and k_{u2} reported in Table 1 for each of the two possible mechanisms. Mechanism A correlates strongly with the experimental data, whereas mechanism B does not, suggesting that the reaction proceeds through a 1:1 complex of oligomer with methyl iodide. Furthermore, at methyl iodide concentrations well below saturation, such as those used in the inhibition experiments, k_2 is given by eq 4,[13] and the parameters obtained from curve fitting of mechanism A provide a value of 0.17 M^{-1} s^{-1} for k_2, in agreement with the previously measured value of 0.159 ± 0.009 M^{-1} s^{-1}.[8]

$$k_2 = k_u K_a \tag{4}$$

In contrast, mechanism B predicts a value of 0.87 M^{-1} s^{-1} for k_2, which deviates significantly from the measured value. We conclude that the agreement between the experimental and predicted reaction rates is much better for mechanism A than for mechanism B.

Effective molarity is defined by eq 5[2] in which k_{intra} is the rate constant for the "intramolecular" reaction and k_{inter} is the rate constant for a reaction proceeding through the same mechanism but lacking enforced proximity of the reagents.

$$\mathrm{EM} = \frac{k_{intra}}{k_{inter}} \tag{5}$$

By using k_u from Table 1 as k_{intra} and k_2 for oligomer **1**[8] as k_{inter}, the EM for methylation of **2** was calculated to be 230 M.[14] Thus, by constraining methyl iodide in close proximity to the pyridine nitrogen, the folded structure of the PE oligomer increases the effective molarity of the reagents, giving rise to the observed rate enhancement.

Conclusions

Competitive inhibition experiments demonstrate the ability of a pyridine-containing PE oligomer to act as a molecular receptor by binding methyl iodide and constraining it within close proximity to the pyridine moiety, thereby accelerating the methylation reaction. The methylation reaction studied in this work requires stoichiometric quantities of the receptor, excluding the oligomer

(13) At concentrations well below saturation, $K_a[M] \ll 1$, so $k_{obs} = k_u K_a[M]$.

(14) EM values for enzymes are frequently $\geq 10^8$ M (ref 1b). However, many synthetic enzyme mimics reported in the literature have EM values of <1 M (ref 1j).

from acting in a catalytic manner. However, it demonstrates how the structural diversity provided by foldable oligomers can lead to the development of a new subset of molecular receptors, and future work will focus on applying these concepts to reactions in which the oligomer has the potential to act catalytically.

Acknowledgment. This research was funded by the Department of Energy, Division of Materials Sciences (DEFG02-91ER45439) through the Frederick Seitz Materials Research Laboratory at the University of Illinois at Urbana−Champaign and the National Science Foundation (CHE 03-45254). J.M.H. thanks the Nelson J. Leonard ACS Division of Organic Chemistry Fellowship, sponsored by Organic Syntheses, Inc.

Supporting Information Available: Experimental procedures for measuring methylation rates of **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO048506D