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An oligomer containing 4-(dialkylamino) pyridine groups incorporated within a bis(trimethylineamine)disiloxane backbone exhibits remarkable catalytic activity in the hydrolysis of p-nitrophenyl alkanoates. p-Nitrophenyl tetradecanoate is the optimum substrate in 1:1 methanol-water at pH 8, 30 °C. The structure of the oligomer's catalytically active conformation was investigated by 2D NOESY experiments. The resultant structure parameters obtained with aggregates of catalyst or catalyst-myristoleic acid complex were used to model the intermolecular interactions of catalyst and substrate. This uniquely informative NOESY distance information may explain the enzyme-like, chain length specificity for catalysis of C-14 fatty acid esters.

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2D NOESY Spectra of a Synthetic Siloxane Oligomer with Selective Esterase Activity

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2D NOESY NMR Spectra of a Synthetic Siloxane Oligomer with Selective Esterase Activity

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April 29, 1992

One of our laboratories has recently shown that an oligomer (MW~ 3500, 1) containing 4-dialkylaminopyridine groups incorporated within a bis-(trimethyleneamine)disiloxane backbone (Figure 1a) has remarkable catalytic activity in the hydrolysis of p-nitrophenyl alkanoates.[1] This oligomer exhibits Michaelis-Menten kinetics for lipophilic substrates and demonstrates enzyme-like specificity for esters derived from acids of moderate chain length ($C_{12} \rightarrow C_{16}$) with p-nitrophenyl tetradecanoate (C_{14}) as the optimal substrate. The origin of this catalytic activity and specificity is, as yet, unknown. In this report we describe a 2D NMR study which provides some clues as to the structure of a substrate model-oligomer complex as well as the origin of the chain-length specificity for substrates.

In an effort to determine the structure of its catalytically "active" conformation, the oligomer 1 was studied in mixed micellar aggregates with product inhibitor surfactants. Myristoleic acid, (cis-9-tetradecanoic acid, 2) (Figure 1b), readily solubilized 1 and presumably formed a mixed micelle or other aggregate in methanol/water (1:1 by volume) with the oligomer.[1–3] The NMR of both the cis and trans forms of 2 as well as other long chain surfactants were comparable but only the results from studies with the cis isomer are reported here. The presence of the double bond has proven to be very useful in allowing us to assign NMR signals in the hydrophobic interior region of the surfactant/oligomer aggregate.

Samples were prepared by adding 5.0 mg (0.02 mmol) of 1 to 0.50 mL CD_3OD, followed by the addition of 8.6 mg (0.038 mmol) of 2 (Aldrich). D_2O (0.50 mL) was then added to make the solution 1:1 in methanol-water. A white colloidal suspension formed immediately. This colloidal solution gave broad ^1H NMR signals for both 1 and surfactant with short T_1 values (see below) consistent with large macromolecular aggregates. Upon standing at room temperature for 48-72 h, the solution clarified. The ^{1}H NMR signals for both surfactant and *1* sharpened considerably with lengthening of the T_1 values consistent with formation of smaller micellar aggregate units. This solution is thermodynamically stable and the complex cannot be separated by centrifugation.

The assignments of the ^{1}H NMR spectrum of the *1*/*2* complex were made using chemical shift information of the components and phase sensitive 2D DQFCOSY[4] and NOESY[5] spectra. The ^{1}H T_1 longitudinal relaxation times were measured using an inversion recovery pulse sequence. There does not appear to be a significant difference between the T_1's of individual protons of *1* and *2* suggesting that they are co-aggregated and tumbling with the same overall correlation time of a single macromolecular complex. The average T_1 of the white colloidal samples is 0.6 s, while the clear, colorless samples show an average T_1 of 0.8 s.

The 2D NOESY spectra of the intermolecular complex are reminiscent of a large, conformationally stable macromolecule. Thus, most NOESY crosspeaks are negative as expected for a macromolecule (Figure 2). Some much smaller positive NOESY crosspeaks also observed are believed to arise from minor, unassociated surfactant or smaller oligomer species dissolved in solution. Crosspeak volumes were integrated from NOESY spectra taken at 50, 100, 200, 250 and 300 ms and NOE buildup curves were determined for each crosspeak. Intramolecular crosspeaks for both *1* and *2* show a nearly linear, rapid NOE buildup for nearby protons as expected by the inverse sixth power dependence on the initial rate of NOE build-up for direct cross-relaxation between protons < 5 Å apart.[6] (Other factors such as the correlation time and time for the cross-relaxation to evolve can also contribute to the NOE). At longer mixing times intramolecular crosspeaks are also observed between protons much further separated, indicating efficient spin diffusion.[6,7] Significantly, *intermolecular* crosspeaks between the *2* and *1* protons are also observed. Some of the buildup curves for these intermolecular proton pairs show a lag or induction period during the initial buildup indicating the effect of two-step or indirect magnetization transfer (i.e., spin diffusion).[7] However, the intermolecular crosspeaks, measured at short, 50 ms mixing time, are quite specific and are therefore not simply due to very fast intramolecular redistribution of the magnetization.[8] At longer mixing time (300 ms), nearly all signals of both *1* and *2* do indeed show crosspeaks to each other (i.e., spin diffusion among all of the protons in the sample is nearly complete). This is expected since most solvent molecules (with only a small residual ^{1}H enrichment) should be excluded from the interior of the intermolecular aggregate and spin-lattice relaxation of the intermolecular aggregate appears to be segregated from the solvent bath.[8,9]

Although this efficient spin diffusion complicates quantitative distance determination between protons, the relative crosspeak volumes in the 50 ms NOESY spectrum provide important qualitative information. Thus, the vinylic protons of the fatty acid appear to be in closest spacial proximity to the siloxane methyl groups. At the same time the siloxane methyl protons are also closest to the N, M and Q methylene protons of *2*. (We do not see crosspeaks to the L and P methylenes at short mixing times, confirming the specificity of these interactions.) Unless the conformation of the surfactant polymer complex is heterogeneous, it is difficult to see how these siloxane methyl protons can be simultaneously close to all of the indicated methylene protons in an extended chain conformation of the surfactant. A ROESY spectrum of the complex was also acquired to rule out the possibility that the intermolecular crosspeaks were due to chemical exchange (data not shown). Since the ROESY crosspeaks had opposite phase to the diagonal they are not attributable to chemical exchange effects.[7]

Within the complex it is expected that the charged carboxylic acid and polar pyridinium rings will be closest to the surface, with the hydrophobic siloxane methyl and other methylenes of both *1* and *2* intertwined in the interior. Based upon the simple siloxane repeating unit of 10 atoms, it is not possible to arrange an extended form for both the oligomer and the surfactant chain (15 atoms). As in simple hydrocarbons, phospholipid bilayers, and long chain hydrocarbon polymers, the anti conformation about the C-C bond is preferred, and most hydrocarbon chains will largely be in a time-averaged extended form with only a few gauche conformations.[10] Importantly, protons on the pyridine ring show intermolecular crosspeaks to the long chain methylene region of the fatty acid. This is inconsistent with the picture of a surface pyridine ring and a single siloxane unit binding to a single long chain fatty acid. One solution to these inconsistencies is suggested in Figure 1, where we have juxtaposed 1.5 siloxane units (15 atoms long) with the 15 heavy atom-long chain of *2*. This arrangement can now explain the simultaneous proximity of siloxane methyls to both the surface and interior protons of the surfactant and in addition explain the proximity of the pyridine protons to the interior methylenes of *2*. This proposed model for intermolecular interactions and association requires that every other pyridine ring is buried within the hydrophobic core of the micelle. Indeed we find that only ca. half of the pyridines in *1* can be titrated with strong acid.

Although other intermolecular NOEs have been observed in mixed micelles[11-13] and mixed polymer-polymer blends at high concentration in non-aqueous solution,[8,14,15] the uniquely informative NOESY distance information that is observed in the siloxane-surfactant complex may explain the remarkable enzyme-like chain length specificity for catalysis of C-14 fatty acid esters. Thus, a shorter or longer chain in a fatty acid substrate may not as readily match the heavy atom chain length of the 15-carbon atom repeating unit shown in Figure 1.

Acknowledgments

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Figure Legends

Figure 1. Structure of myristoleic acid, 1 (top) and disiloxane oligomer, 2 (bottom). The repeating oligomeric unit is shown in parentheses. Protons are labeled and arrows represent some of the intramolecular and intermolecular NOESY crosspeaks observed for the mixed micellar aggregate.

Figure 2. Expanded 2D pure absorption phase NOESY NMR spectrum (50 ms mixing time) of the myristoleic acid/disiloxane oligomer complex in CD_3OD/D_2O (1:1). Spectrum taken on a Varian VXR 500 MHz spectrometer at 22° C. Peaks marked with X represent impuritites or smaller unassociated components.

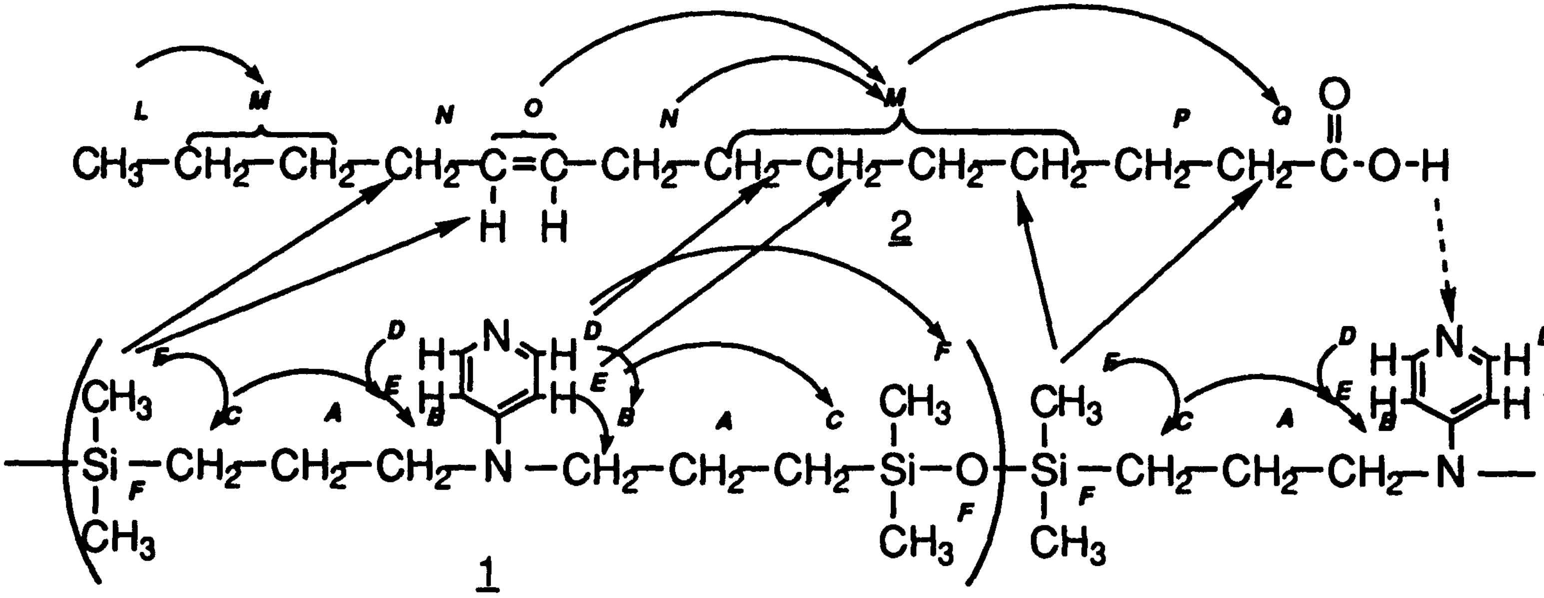
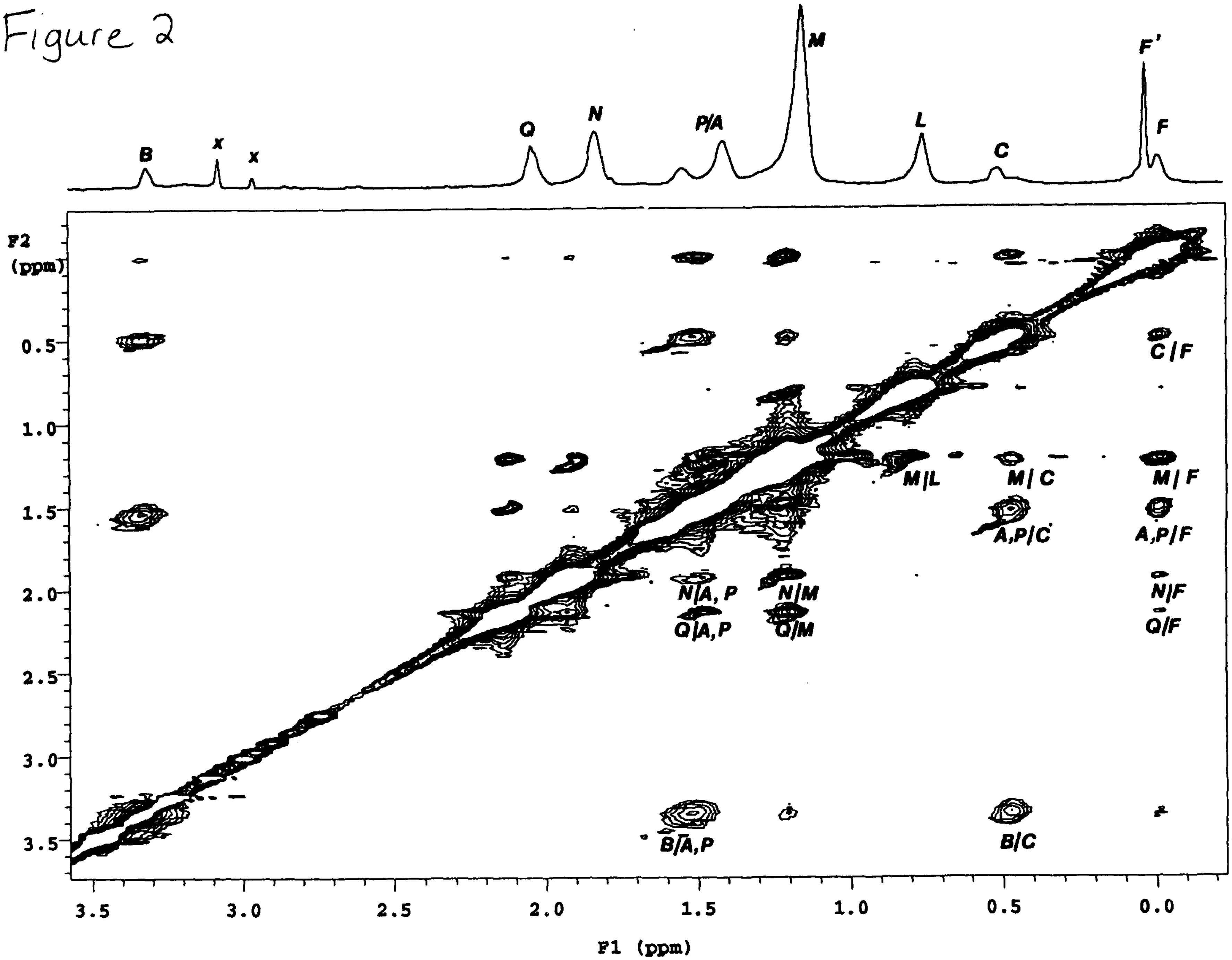


Figure 1

Figure 2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