Folding Energetics and Dynamics of Macromolecules in the Gas Phase: Alkali Ion-Cationized Poly(ethylene terephthalate) Oligomers

Jennifer Gidden,[†] Thomas Wyttenbach,[†] Joseph J. Batka,[†] Patrick Weis,[†] Anthony T. Jackson,[‡] James H. Scrivens,[‡] and Michael T. Bowers*,[†]

*Department of Chemistry, University of California
Santa Barbara, California 93106
ICI Research and Technology Centre, P.O. Box 90
Wilton, Middlesborough, Cleveland, U.K. TS90 8JE*

*Received August 31, 1998
Revised Manuscript Received December 2, 1998*

The question of the energetics, structure, and dynamics of macromolecular folding is one that cuts across the chemical horizon. The problem is probably most apparent in the biochemistry field, where protein folding has become a forefront issue.[1] Historically, structural experiments have been carried out in the condensed phase, usually using X-ray crystallography or, more recently, multidimensional NMR techniques.[2] Condensed-phase experiments can sometimes approach the actual environment for the active system, but the downside to this approach is that the results can often be strongly medium dependent, and hence the effects of the medium must be thoroughly understood if intrinsic structural or energetic information is to be obtained.

Mass spectrometry has long played an important role in determining the structural and energetic properties of organic molecules and small macromolecules and clusters. In this past decade, however, dramatic progress has been made on larger systems, primarily due to the emergence of matrix-assisted laser desorption/ionization (MALDI)[3] and electrospray ionization (ESI)[4] as convenient high-brightness sources. Determination of protein and RNA/DNA sequences (primary structure) have become routine, and mass spectrometric methods are often the technique of choice due to their very high sensitivity. Unfortunately, determination of higher order structure remains a difficult and complex issue.

One method that has recently emerged that addresses the molecular conformation issue is the ion mobility-based "ion chromatography" (IC) technique.[5] While IC was developed for application to atomic ions and clusters,[6,7] of late it has been applied successfully to both smaller[8−10] and larger sized[11−13] biopolymers

Table 1. Experimental and Theoretical Cross Sections (Å) for Na⁺((PET)n Oligomers

n	expt[a]	theory[a]
2	135	133
3	175	164 (closed)
		182 (open)
4	203	198

[a] Experiments done at 300 K. Experimental uncertainty is about 1%. Theoretical uncertainty is ± 2 Å².

as well as synthetic polymers.[14−16] The basis of the method is the measurement of an arrival time distribution (ATD) of a short pulse of mass-selected ions that have drifted through a cell containing an inert collision gas under the influence of a weak electric field. From the ATD, the ion mobility is accurately determined and using kinetic theory[17] a three-dimensional collision cross section obtained. Theoretical methods can then be used to generate candidate structures and cross sections, allowing structural (i.e., conformational) identification of the isomers.[18,19]

In this communication, we apply ion chromatography methodology to smaller sized poly(ethylene terephthalate) (PET) oligomers. PET is an important industrial polymer and the primary constituent in DACRON fiber and most molded plastic laboratory containers. PET has both flexible and rigid portions, and the
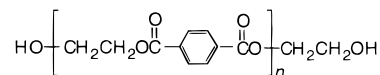
folding dynamics, energetics, and resulting structures could well be complex. It is our intention to explore how this complexity affects the dynamics of folding of this interesting species.

We have done IC experiments on PET oligomers for $n = 2-7$, cationized by the alkali ions M⁺ = Li⁺, Na⁺, K⁺, and Cs⁺. In this work, we will focus on $n = 3$ and M = Na. Molecular mechanics calculations were done using the AMBER 4.0 suite of programs[20] and generation of candidate structures using annealing protocols described elsewhere.[8] These protocols are designed to yield low-energy conformers. In each instance, a scatter plot was generated for the M⁺(PET)n oligomer of interest, consisting of the cross sections of 100 structures plotted against their corresponding energies. An example is given in the Supporting Information (Figure 1S).

At 300 K, a single ATD peak was observed for Na⁺(PET)n for all values of n. The cross sections derived from the peaks for $n = 2$, 3, and 4 are given in Table 1, along with the cross sections of the low-energy conformers obtained from the molecular mechanics simulations. For $n = 2$ and 4, there is excellent agreement between experiment and theory. For $n = 2$, the calculations indicate a very simple structure. The oligomer chain bends so that the benzene rings "π-stack". The Na⁺ ion coordinates with both terminal hydroxyl oxygens and the two terminal carbonyl oxygens, yielding a tightly folded pancake structure. A similar structure is obtained for $n = 4$.

† University of California, Santa Barbara.
‡ ICI Research and Technology Center.
(1) Creighton, T. E. *Proteins: Structures and Molecular Properties*, 2nd ed.; W. H. Freeman: New York, 1993.
(2) For a detailed discussion and references, see: Zolotov, Y. A., Ed. *Macrocyclic Compounds in Analytical Chemistry*; Wiley-Interscience: New York, 1997.
(3) Hillenkamp, F.; Karas, M.; Beavis, R. C.; Chait, B. T. *Anal. Chem.* **1991**, *63*, 1193A.
(4) Fenn, J. B.; Mann, N.; Meng, C. K.; Wong, S. F. *Mass Spectrom. Rev.* **1990**, *9*, 37.
(5) Bowers, M. T.; Kemper, P. R.; von Helden, G.; van Koppen, P. A. M. *Science* **1993**, *260*, 1446.
(6) Kemper, P. R.; Bowers, M. T. *J. Phys. Chem.* **1991**, *95*, 5134.
(7) von Helden, G.; Hsu, M.-T.; Kemper, P. R.; Bowers, M. T. *J. Chem. Phys.* **1991**, *95*, 3835.
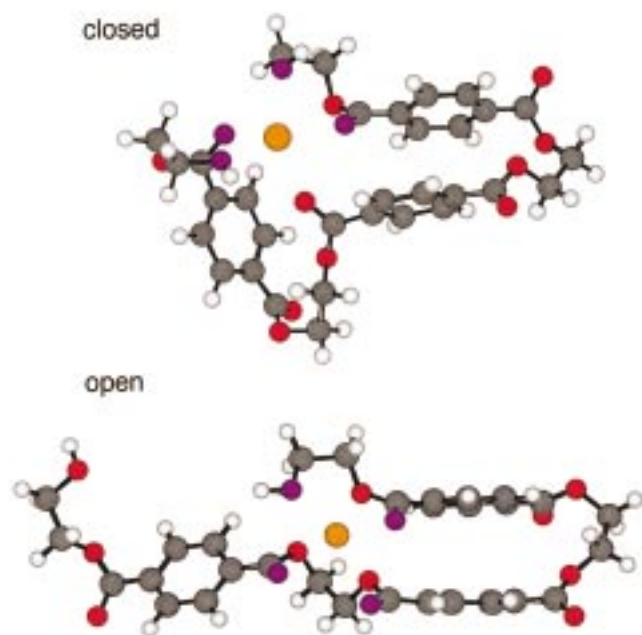(8) Wyttenbach, T.; von Helden, G.; Bowers, M. T. *J. Am. Chem. Soc.* **1996**, *118*, 8355.
(9) Lee, S.; Wyttenbach, T.; Bowers, M. T. *Int. J. Mass Spectrom. Ion Processes* **1997**, *167/168*, 605.
(10) Wyttenbach, T.; Bushnell, J. E.; Bowers, M. T. *J. Am. Chem. Soc.* **1998**, *120*, 5098.
(11) Clemmer, D. E.; Hudgins, R. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **1995**, *117*, 10141.
(12) Shelimov, K.; Jarrold, M. F. *J. Am. Chem. Soc.* **1996**, *118*, 10313.
(13) For a recent review of applications of ion mobility measurements to clusters and biomolecules, see: Clemmer, D. E.; Jarrold, M. F. *J. Mass Spectrom.* **1997**, *32*, 577.

(14) von Helden, G.; Wyttenbach, T.; Bowers, M. T. *Science* **1995**, *267*, 1483.
(15) von Helden, G.; Wyttenbach, T.; Bowers, M. T. *Int. J. Mass Spectrom. Ion Processes* **1995**, *146/147*, 349.
(16) Wyttenbach, T.; von Helden. G.; Bowers, M. T. *Int. J. Mass Spectrom. Ion Processes* **1997**, *165/166*, 377.
(17) Mason, E. A.; McDaniel, E. W. *Transport Properties of Ions in Gases*; Wiley: New York, 1988.
(18) von Helden, G.; Hsu, M.-T.; Gotts, N.; Bowers, M. T. *J. Phys. Chem.* **1993**, *97*, 8182.
(19) Gotts, N. G.; von Helden, G.; Bowers, M. T. *Int. J. Mass Spectrom. Ion Processes* **1995**, *149/150*, 217.
(20) Pearlman, D. A.; Case, D. A.; Caldwell, J.; Seibel, G. L.; Singh, U. C.; Weiner, P.; Kollman, P. A. *AMBER 4.0*; University of California, San Francisco, CA 1991.

Figure 1. Typical low-energy structures of the closed and open forms of Na$^+$(PET)3. The orange ball is the Na$^+$ ion, coordinated oxygens are purple, noncoordinated oxygens red, carbons gray, and hydrogens white.
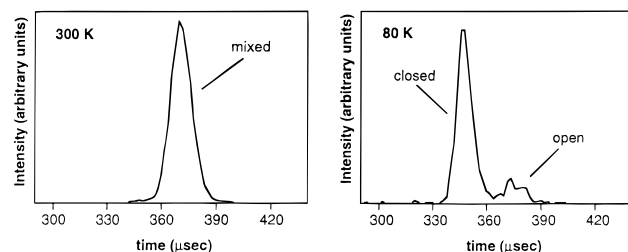
**Figure 2.** Arrival time distributions for Na$^+$(PET)3 at 300 and 80 K. The absolute values of the arrival times cannot be compared between the two experiments due to different experimental conditions.

The situation for $n = 3$ is more complex. In this case the first two monomer units π-stack to form a structure very similar to the 2-mer. However, the third monomer does one of two things: either it simply attaches with the terminal hydroxyl group remote from the Na$^+$ ion (the open form), or it contorts in such a way that the terminal hydroxyl oxygen bends back and coordinates with the Na$^+$ ion (the closed form) (Figure 1). Theory indicates that these two forms are very close in energy with the closed form about 1 kcal/mol more stable.[21] Theory also predicts their cross sections to be about 10% different, a difference that should be easily resolved under our experimental conditions. However, the 3-mer ATD shows only one peak, and the resulting experimental cross section falls between the theoretical values of the open and closed forms (Table 1).

The results above suggest that the two isomeric forms of Na$^+$-(PET)3 might be in dynamic equilibrium at 300 K. Consequently, we cooled the mobility cell to 80 K to see if this putative isomerization could be frozen out. The results are given in Figure 2. The fact that the two peaks are clearly resolved indicates that the average interconversion rate between the two isomers at 80 K is slower than the rate of passage of the ions through the mobility cell.

Kinetic theory[22] was adapted to our case and used to fit a series of experimental ATDs taken between 110 and 190 K (Figure 2S, Supporting Information). The only variable parameters in the fits

(21) See Supporting Information.
(22) Gatland, I. R. *Case Stud. At. Phys.* **1974**, *4*, 369.
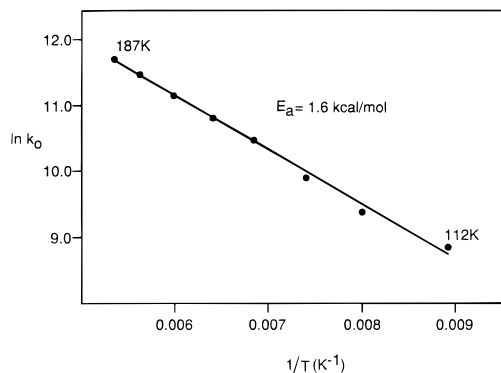
Figure 3. Plot of ln k_o versus $1/T$ for the isomerization of Na$^+$(PET)3 from open to closed.
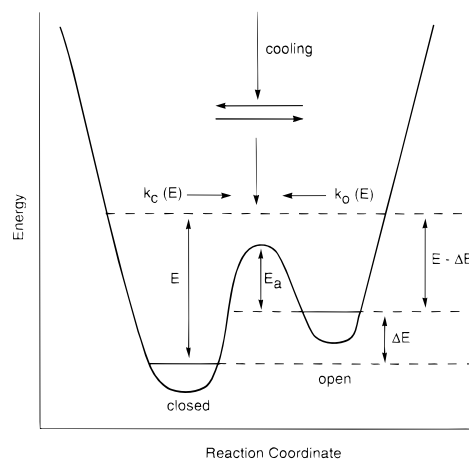
Figure 4. Schematic reaction coordinate diagram for the isomerization of Na$^+$(PET)3.

were the open-to-closed rate constant, k_o, and the closed-to-open rate constant, k_c. Hence, we were able to extract $k_o(T)$ over the temperature range 110–190 K. The data are plotted in Figure 3 as ln k_o versus T^{-1}, with the slope yielding an Arrhenius activation energy of $E_a = 1.6$ kcal/mol for the isomerization.

A useful simplified reaction coordinate diagram is given in Figure 4. At 300 K, the average energy of the system is sufficiently far above the barrier that rapid interconversion takes place. As the system cools, this average energy approaches the barrier height. When it passes below the barrier, interconversion ceases. The observed relative peak intensities in the ATD at 80 K are very good approximations of the fractional abundances of the closed and open forms at energy $E = E_a + \Delta E$. The densities of states of the two species were calculated at various values of ΔE until the experimental abundances were reproduced. This corresponded to a value of $\Delta E = 0.5$ kcal/mol, in good agreement with the molecular mechanics estimate of 1.0 kcal/mol.

In summary, the power of the IC method to temporally and spatially resolve different conformers is clearly demonstrated. Not only are the two dominant low-energy conformers of Na$^+$(PET)3 observed, but their relative energies and isomerization barriers are also obtained. To our knowledge, this is the first such determination for macromolecules of this complexity in the gas phase.

Supporting Information Available: Plot of the cross section versus energy of 100 structures of Na$^+$(PET)3 generated by an annealing procedure described in ref 8, and a series of arrival time distributions of Na$^+$(PET)3 at 100, 112, and 125 K. This material is available free of charge via the Internet at http://pubs.acs.org.

JA9831047