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Collision cross sections of gas phase DNA ions

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Abstract

Collision cross sections of negative ions of a 28-, 40- and 55-mer of single stranded DNA have been measured by an energy loss method, and compared to collision cross sections of proteins of nearly the same molecular weight—ubiquitin, cytochrome *c* and apomyoglobin, respectively. The oligonucleotides produce negative charge states in electrospray ionization (ESI) similar to the positive charge states produced by the proteins denatured in solution. Cross sections for deoxynucleotide ions increase with charge state in a manner similar to those of protein ions. However, for a given molecular weight and charge state, the cross sections of the oligodeoxynucleotide ions are about 22% lower than those of the proteins. (Int J Mass Spectrom 219 (2002) 161–170)

Keywords: Collision cross sections; DNA ions; Gas phase; Protein ions

1. Introduction

The advent of electrospray ionization (ESI) [1] has allowed the formation of gas phase ions of intact biomolecules such as peptides, proteins and oligonucleotides. This has led to a revolution in the applications of mass spectrometry to life sciences. It has also stimulated interest in the structures of these ions, particularly proteins and peptides [2], because the folding of these ions can be studied under conditions where the ions are completely free of solvent. This gives insights to the intrinsic intramolecular interactions that contribute to conformations in solution.

Of the various methods used to study gas phase bio-ions, measurements of collision cross sections give the most direct insights to folding. Collision cross sections are also important for understanding the transport of ions through relatively high pressure regions of the sampling interface of an ESI mass spectrometer system [3a,b] or the collision cell of an MS/MS system [3c]. Cross sections have been determined in ion mobility experiments [1,2], energy loss experiments [4], experiments that examine impact damage to graphite surfaces caused by ions [5], and high energy collisions [6]. Of these methods, ion mobility has the highest resolution and can, in some cases, separate ions of a biopolymer that have the same mass to charge ratio but different conformations. The energy loss method is lower in resolution, but is easily implemented on some triple quadrupole MS/MS systems

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and can be applied to fragile ions such as non-covalent complexes [7]. Neither method provides a direct measurement of the ion projection cross section. This is a predicted value based on modeled interactions between the analyte molecule and background gas. The manner in which energy and momentum transfer is modeled for a collision has a significant effect on the calculated cross section. This is particularly true when comparing values determined from ion mobility and energy loss experiments since the background gas pressures differ significantly. It has been shown that cross sections determined by mobility and energy loss experiments agree within a few percent provided both experiments are interpreted with a collision model that corresponds to diffuse scattering [4b].

Most cross section measurements to date have concerned peptide and protein ions [2]. There has been only one report of cross sections for oligodeoxynucleotide ions. Hoaglund et al. [8] described cross sections of anions of the 10-mer of polythymine, and showed that cross sections increase with charge state in a manner similar to protein ions. Modeling showed the charges were distributed evenly along the phosphodiester backbone to minimize Coulomb repulsion, as would be expected.

An interesting issue in the mass spectrometry of nucleic acids is the severe fall-off in signal intensity with increasing mass, observed in some MALDI and ESI experiments [9–11]. This fall-off is not generally observed for proteins. The fall-off in signal intensity limits the analysis of dideoxy sequencing reaction products by mass spectrometry. One factor which might play a role in the phenomenon is differences in the transfer efficiency of protein and DNA ions from the ionization region to the mass analyzer. Such differences, if they exist, might stem from differences in collision cross sections. Investigating this issue was the main motivation for the present work.

In this paper, we report collision cross sections for anions of 28-, 40- and 55-mer single stranded oligodeoxynucleotides and compare them to collision cross sections of proteins of nearly the same molecular weights—ubiquitin, cytochrome *c* and apomyoglobin, respectively. The oligonucleotides produce negative charge states in ESI similar to the positive charge states produced by the proteins denatured in solution. Cross sections for the deoxynucleotide anions increase with charge in a manner similar to those of the protein ions. However, for a given molecular weight and charge state, the cross sections of the oligodeoxynucleotide ions are about 22% lower than those of the proteins.

2. Experimental

Collision cross sections were determined by measuring the axial energy loss of ions passing through low density argon in the collision cell of a triple quadrupole system, as described previously [4b]. Ions formed by ESI passed through a dry nitrogen curtain gas, a sampler and skimmer, and into a radio frequency (RF) only quadrupole, operated at a pressure of 7×10^{-3} Torr, where their axial energies and energy spreads were cooled to ca. 1 eV by collisions. Ions then passed through an RF only quadrupole and entered a collision cell with an energy of 10 eV per charge. At this collision energy, no fragmentation of the ions was observed. The cell argon pressure was typically varied up to 1.4×10^{-3} Torr. The energy losses from collisions with argon were measured by determining stopping curves with the rod offset of the mass analyzing quadrupole after the collision cell. The continuum background in the spectra was subtracted from the peak intensity. The energies at the cell exit, E, measured at different number densities of argon in the collision cell, n, were fit to

$$\frac{E}{E^0} = \exp\left(-\frac{C_\mathrm{d} n m_2 \sigma l}{m_1}\right) \quad (1)$$

where E^0 is the axial energy at the cell entrance, C_d a drag coefficient for diffuse scattering, n the number density of gas in the cell, m_2 the collision gas mass, σ the collision cross section, l the cell length and m_1 is the ion mass [4b]. For the DNA samples, negative ions were measured and for proteins, positive ions.

The sequence and molecular weights of the single stranded DNA samples are shown in Table 1. These

Table 1
DNA sequences and molecular weights

	Sequence	MW
28-mer	5′-TGT AAA ACG ACG GCC AGT GCC AAG CTT G-3′	8,622.6
40-mer	5′-TGT AAA ACG ACG GCC AGT GCC AAG CTT GCA TGC CTG CAG G-3′	12,331.0
55-mer	5′-TGT AAA ACG ACG GCC AGT GCC AAG CTT GCA TGC CTG CAG GTC GAC TCT AGA TTA A-3′	16,985.0

sequences correspond to a region in the cloning vector M13mp18 [12]. All DNA samples were obtained purified by reverse-phase HPLC from Integrated DNA Technologies, Inc. (Coralville, IA) and quantified by UV absorbance at 260 nm. The oligodeoxynucleotides were diluted in a buffer of 1:1 H_2O:MeOH, 400 mM 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (adjusted to pH 7 with triethylamine) [13] to a concentration of 20 μM. Bovine red cell ubiquitin (76 residues, MW 8565), horse heart cytochrome *c* (104 residues, MW 12,318) and horse heart holomyoglobin (153 residues, MW 17,568) were from Sigma Chemical Company. Protein solutions were: ubiquitin, 50 μM in 49% CH_3CN, 49% H_2O, 2% acetic acid; cytochrome *c*, 10 μM in 50% CH_3OH, 50% H_2O adjusted to pH 3 with hydrochloric acid; myoglobin, 10 μM in 50% CH_3CN, 50% H_2O, 0.1% acetic acid.

HPLC grade acetonitrile, methanol, acetic acid and hydrochloric acid were from Fisher Chemical Company (Nepean, ON). HFIP, 99.8% purity, was from Aldrich Chemical Company. The curtain gas, UHP grade nitrogen, manufacturers' stated purity (99.999%) and Linde grade argon, manufacturers' stated purity 99.9995%, were from Praxair (Mississauga, ON, Canada).

3. Results and discussion

Negative ion mode mass spectra of the 28-, 40- and 55-mer are shown in Fig. 1A–C, respectively and positive ion mode mass spectra of ubiquitin, cytochrome *c* and myoglobin are shown in Fig. 2A–C, respectively. These proteins were chosen because they have molecular weights very similar to the oligonucleotides and do not contain disulphide bonds. The proteins were denatured in solution and so produced relatively high charge states [14]. For the same reason, holomyoglobin produced ions of apomyoglobin, MW 16,951 [15]. It is interesting to note that the DNA samples produce negative charge states in ESI that are similar to those produced in positive mode by denatured proteins of the same molecular weight. The average charges on ubiquitin, cytochrome *c* and apomyoglobin are +10.5, +14.6 and +18.0 and the average charge on the 28-, 40- and 55-mer are −10.3, −14.3 and −21.3, respectively. (The average charge is calculated as $\sum_i N_i I_i / \sum_i I_i$ where N_i is the number of charges on a peak in the spectrum and I_i is the relative intensity of the peak.) With proteins, protonation is limited primarily to the N-terminus and basic side chains. With oligodeoxynucleotides, each nucleotide contains a phosphate group and can in principle contribute one negative charge. For the DNA samples, the numbers of charges per nucleotide range from about 0.2 to 0.5 with an average of 0.37, an average of one charge for every 2.7 nucleotides. For the proteins there is one charge for about every 7.5 residues.

It is well established that native conformations of proteins in solution produce low charge states in ESI and high charge states of proteins are usually formed when a protein unfolds in solution [14–17]. Little work has been done to investigate such relationships for nucleic acids. The high charge states observed for these DNA samples suggest that they have loose unfolded conformations in solution, comparable to those of denatured proteins.

Collision cross sections of ions of the 28-mer and ubiquitin are shown in Fig. 3, of ions of the 40-mer

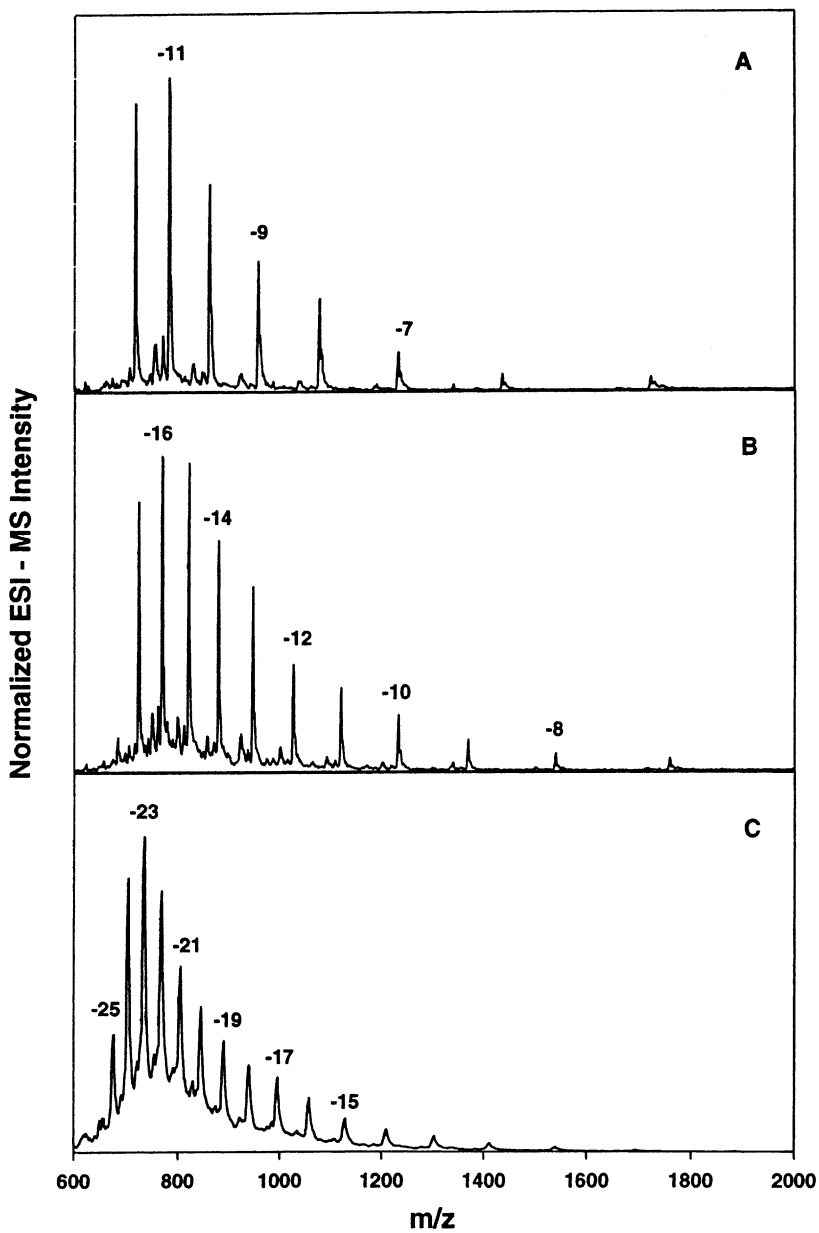

Fig. 1. Mass spectra of (A) the 28-mer; (B) the 40-mer; (C) the 55-mer. The numbers denote the charge states of the ions.

and cytochrome *c* in Fig. 4 and of ions of the 55-mer and apomyoglobin in Fig. 5. The DNA cross sections in Figs. 3–5 are the average of three measurements over several months and the error bars are the relative standard deviations. Collision cross sections for the proteins were measured once and so statistical uncertainties are not shown. However, the cytochrome *c* and apomyoglobin cross sections measured here agree within a few percent with those measured here previously by the same method [4b]. Ion mobility

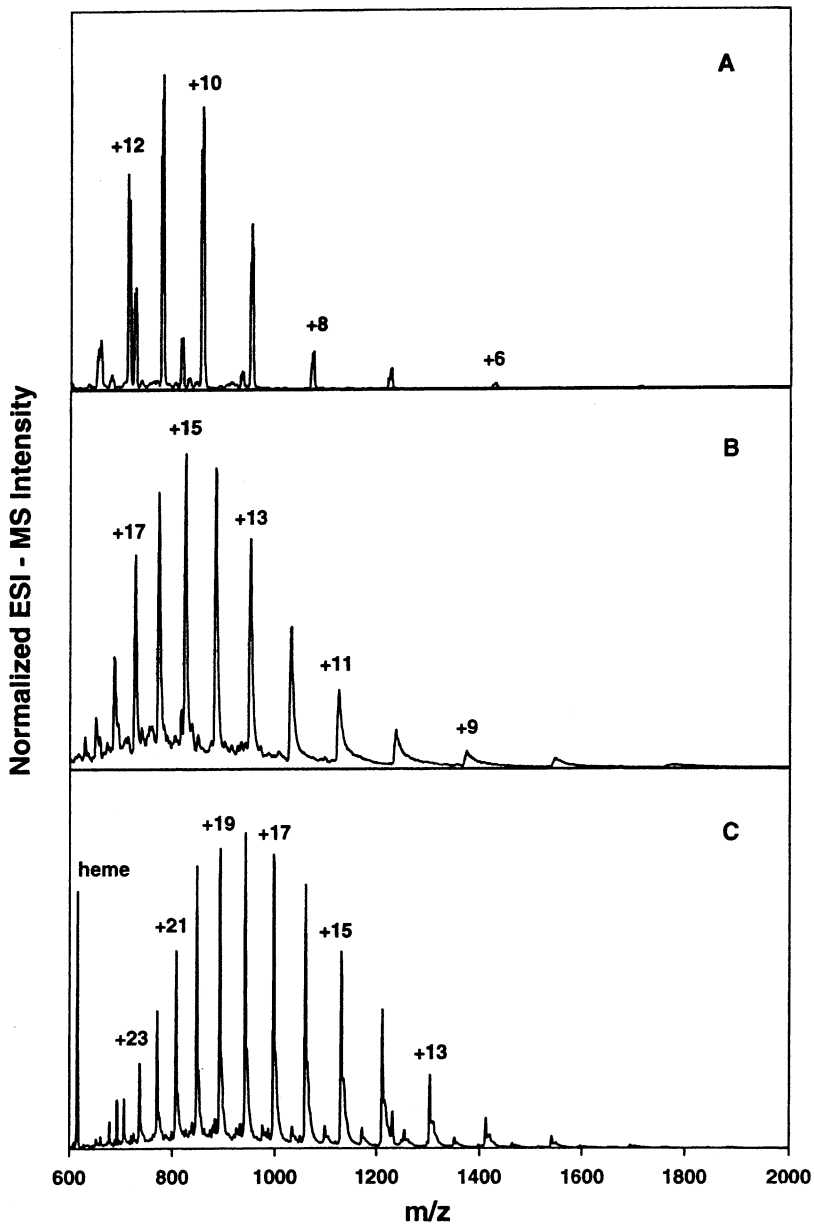

Fig. 2. Mass spectrum of (A) ubiquitin; (B) cytochrome *c*; (C) myoglobin. The numbers denote the charge states of the ions.

experiments have been used to determine cross sections for ubiquitin [18], cytochrome *c* [19] and apomyoglobin [20]. In each case, the reported cross sections were determined from the mobility model for hard spheres, i.e., collisions were treated as hard sphere collisions. It was argued in [4b] that a more realistic model involves "diffuse" scattering. Because the average drag force on an ion with diffuse scattering is greater by a factor of 1.35, to obtain the projection area with diffuse scattering the hard

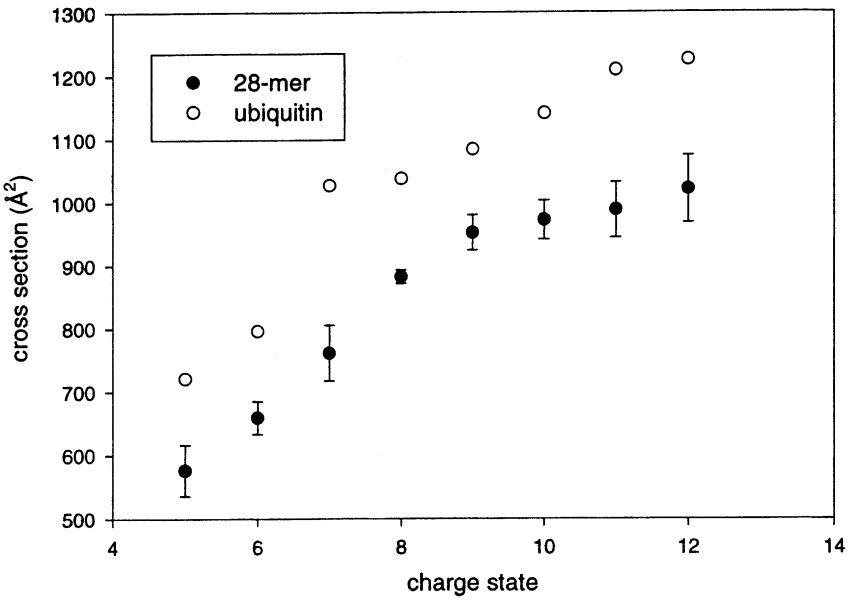

Fig. 3. Collision cross sections vs. charge state of ions of ubiquitin and 28-mer.

sphere cross sections should be reduced by a factor of 0.74. This produces results similar to the "exact hard spheres" model, which requires that cross sections determined by the hard spheres equation be reduced by more than 20% [20]. When the hard sphere cross sections of [15–17] are reduced by a factor of 0.74, the cross sections for ubiquitin in Fig. 3 are found to be 0.96 ± 0.07 on average of those determined by

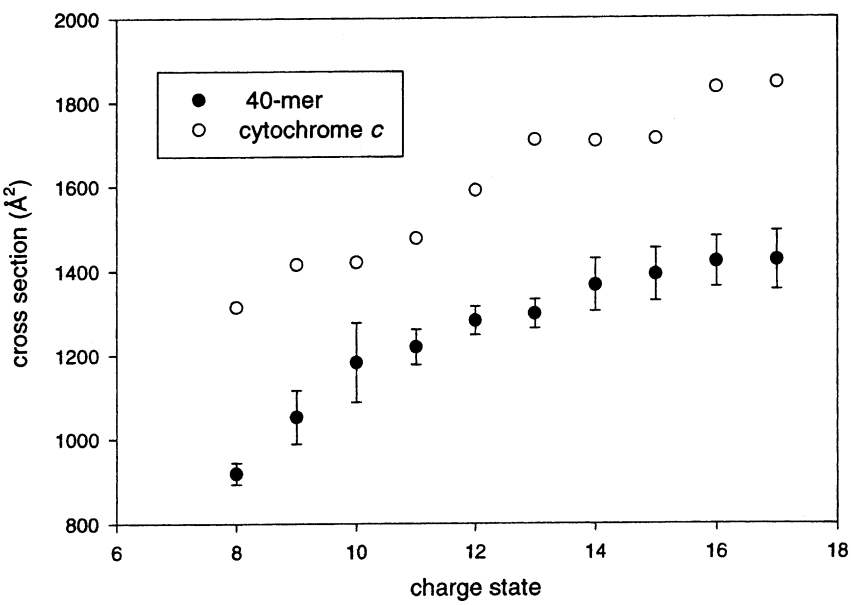

Fig. 4. Collision cross sections vs. charge state of ions of cytochrome *c* and 40-mer.

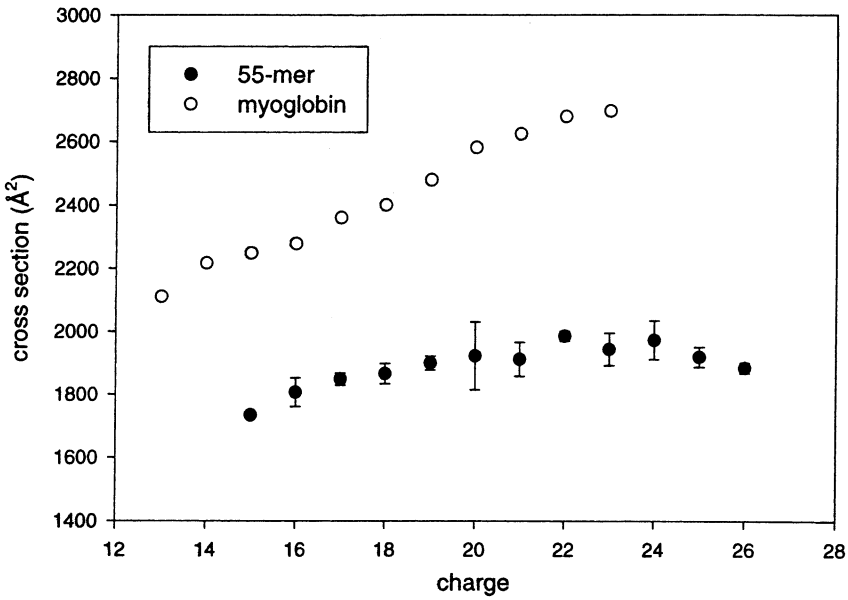

Fig. 5. Collision cross sections vs. charge state of ions of apomyoglobin and 55-mer.

mobility [18]. Averaged over charge states, the cross sections of cytochrome *c* in Fig. 4 are 0.91 ± 0.04 of those determined by mobility [19] and the cross sections of apomyoglobin in Fig. 5 are 0.94 ± 0.01 of those determined by mobility [20]. For the charge states +5, +6, +7, +8 of ubiquitin and +8, +9 of cytochrome *c*, where multiple conformations were detected by mobility, the cross sections here are closest to those of the most compact conformers. The small differences between the energy loss and mobility experiments could easily be attributed to differences in the scattering models used or to differences in the collision dynamics at the low and intermediate energies used in ion mobility and energy loss experiments, respectively. If, for example, a factor of 0.69 is used to correct the hard sphere cross sections (as suggested by Epstein's scattering model [4b]) the energy loss cross sections and mobility cross sections agree within a few percent. The dynamics of scattering of protein ions by Ar or He is not fully understood, and this introduces an uncertainty in converting energy loss or mobility measurements to projection areas or collision cross sections [2a,4b,20,21].

In Figs. 3–5, it is seen that oligonucleotide ions of a given charge and molecular weight have significantly smaller collision cross sections than positive ions of proteins of the same molecular weight. The difference cannot be attributed to the difference in ion polarity. Negative ions of these proteins have similar cross sections to positive ions of the same charge state [2b]. Averaged over charge states, the cross sections of the 28-mer are 0.824 ± 0.041 of the ubiquitin cross sections, the cross sections of the 40-mer are 0.782 ± 0.041 of the cross sections of cytochrome *c* and the cross sections of the 55-mer are 0.758 ± 0.025 of the cross sections of apomyoglobin. There is a slight but significant decrease in the DNA cross sections relative to the proteins as the molecular weights increase. The cross section of the crystal structure of myoglobin has been estimated [20] as 1768 $Å^2$, a value close to those measured here for both the protein and 55-mer ions.

In principle, the difference between the protein and DNA cross sections could be caused by differences in the collision dynamics of these species. The diffuse scattering model used to calculate cross sections

corresponds to highly inelastic collisions. (The evidence that protein–argon collisions are highly inelastic at collision energies near those used in the energy loss experiments is reviewed in [7b].) If collisions of ions of oligodeoxynucleotides with Ar were completely elastic (hard spheres) the drag coefficient in Eq. (1) should be calculated for specular or elastic collisions. For the DNA ions this would decrease the value of C_d by factors between 18% for the low charge states to 12% for the high charge states, so that

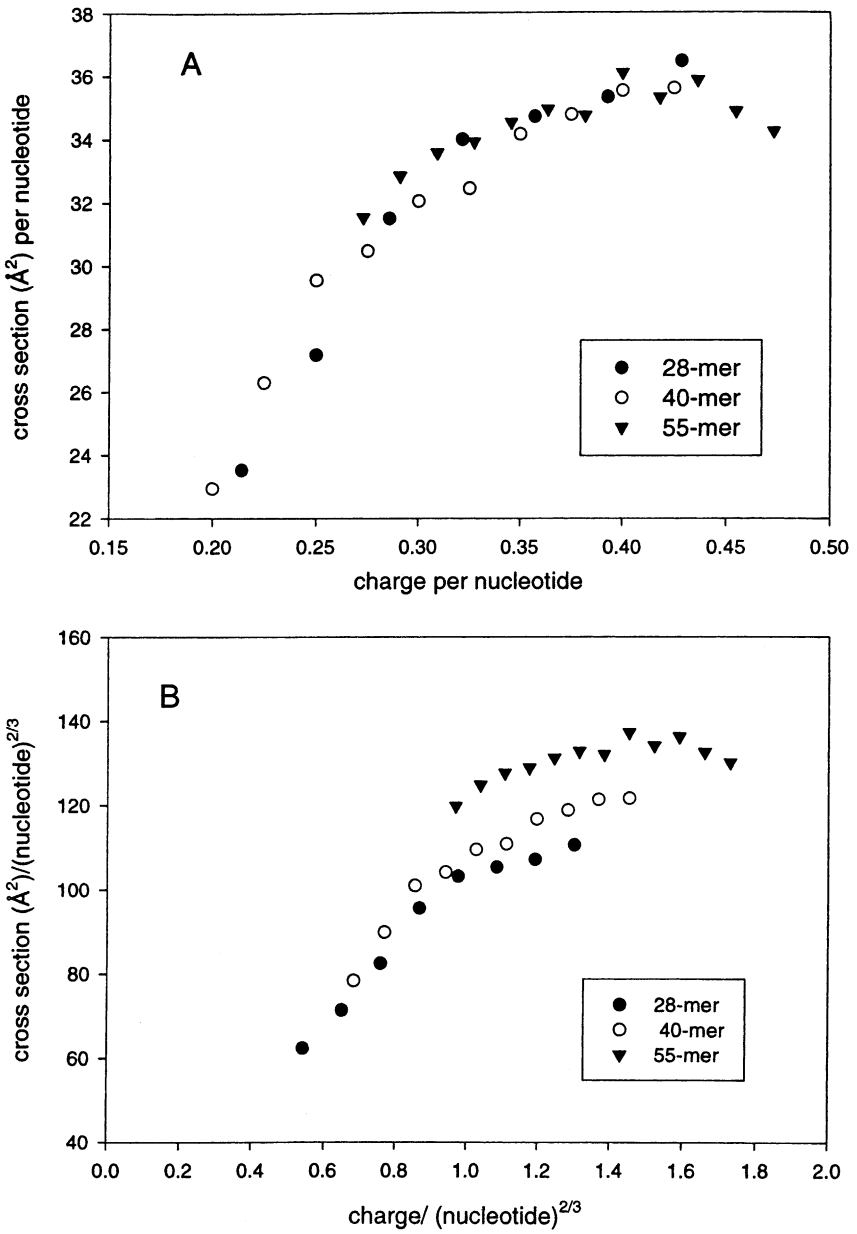

Fig. 6. (A) Cross section per nucleotide vs. charge per nucleotide for ions of the 28-, 40- and 55-mer; (B) cross section/(number of nucleotides)$^{2/3}$ vs. charge/(number of nucleotides)$^{2/3}$ for ions of the 28-, 40- and 55-mer.

cross sections calculated from Eq. (1) would *increase* by the same amount. This would reduce but not eliminate the differences in cross sections between proteins and DNA ions. There is, however, no reason to believe that oligodeoxynucleotide ions would have completely elastic collisions while protein ions had highly inelastic collisions at the same collision energies.

Shelimov and Jarrold, in a study of apomyoglobin ions, proposed that if high charge state ions from different proteins have string-like structures, the cross section per residue should be a universal function of the number of charges per residue [20]. They noted that high charge states of cytochrome *c* and apomyoglobin showed this behavior, even though the cross sections were significantly smaller than those calculated for the fully stretched out proteins. Conversely, if the ions had globular or approximately spherical structures a plot of cross section/(number of residues)$^{2/3}$ should be a universal function of charge/(number of residues)$^{2/3}$. Low charge states of cytochrome *c* and apomyoglobin showed this behavior. Plots showing both the cross section per nucleotide vs. charge per nucleotide and cross section per (nucleotide)$^{2/3}$ vs. charge per (nucleotide)$^{2/3}$ for the DNA ions studied here are shown in Fig. 6A and B, respectively. It is seen that the higher charge state ions from oligonucleotides of varying lengths exhibit a smaller deviation from one another in the plots which are normalized per nucleotide (Fig. 6A) than in the plots which are normalized per (nucleotide)$^{2/3}$ (Fig. 6B). The opposite is true for the lower charge states. Based on the work of Shelimov and Jarrold, this implies that the lower charge states have a globular or spherical conformation while the higher charge states have a non-globular or extended conformation.

As mentioned in the introduction a main driving force for this work was to see if the fall-off in signal intensity with increasing mass as observed in some MALDI and ESI experiments was due to differences in the transfer efficiency of protein and DNA ions from the ionization region to the mass analyzer. Such differences if they exist, might stem from differences in collision cross sections. The results in Figs. 3–5 show that nucleic acids behave similarly to proteins. The relatively small differences in cross sections seen here are unlikely to explain the losses of ion signal for high mass polynucleotide ions.

Determining the structures of gas phase polynucleotides may be an even greater challenge than that of determining structures of gas phase proteins, because the solution structures of polynucleotides are often less well understood. Secondary structure prediction for any given sequence relies on energy minimization algorithms. Due to the constraints of current mathematical models and the uncertainties in the thermodynamic parameters employed, the ability to accurately predict polynucleotide secondary structure is limited, making comparisons of solution and gas phase behavior more difficult [22]. However, single stranded polynucleotides that are self-complementary, studied with additional methods such as H/D exchange, may allow further insights into the gas phase ion structures in future studies.

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