Counting Ions around DNA with Anomalous Small-Angle X-ray Scattering

Suzette A. Pabit, Steve P. Meisburger, Li Li, Joshua M. Blose, Christopher D. Jones, and
Lois Pollack*

School of Applied and Engineering Physics, Cornell University, Ithaca, New York 14853, United States

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Abstract: The majority of charge-compensating ions around nucleic acids form a diffuse counterion "cloud" that is not amenable to investigation by traditional methods that rely on rigid structural interactions. Although various techniques have been employed to characterize the ion atmosphere around nucleic acids, only anomalous small-angle X-ray scattering (ASAXS) provides information about the spatial distribution of ions. Here we present an experimentally straightforward extension of ASAXS that can be used to count the number of ions around nucleic acids.

The number and spatial distribution of small, positively charged ions around highly negatively charged DNA or RNA provide entropic contributions to the free energy of binding in the cell.[1] Thus, salt concentrations and condensed counterions greatly affect the conformation, stability, and binding affinity of nucleic acids.[2-4] Although much attention has focused on the study of specifically bound charged molecules (e.g., bound ions, polyamines, charged protein surfaces), the majority of charge-compensating ions form a diffuse counterion "cloud"[5] that is not amenable to investigation by traditional methods such as X-ray crystallography or ligand-binding chemistry. Although various techniques have been employed to characterize the ion atmosphere around nucleic acids, only anomalous small-angle X-ray scattering (ASAXS) provides information about the spatial distribution of ions.[6] In fact, we recently used ASAXS to highlight differing ion distributions around comparably sequenced double-stranded DNA and RNA helices.[7]

Here we present an experimentally straightforward extension of ASAXS that can be used to count the number of ions around nucleic acids while measuring the ion−nucleic acid spatial distribution. The technique is simple and in principle can easily be implemented in any energy-tunable SAXS beamline with an energy resolution appropriate for ASAXS or multiple-wavelength anomalous diffraction (MAD) experiments. Application of this technique requires absolute calibration of two experimental parameters: scattering intensities and changes in the near-edge scattering factors resulting from resonant effects. These quantities can be obtained readily from a variety of calibration standards.[8,9] Most critically, ion counting via ASAXS does not rely on computing small differences between ion numbers in two solutions (e.g., one with and the other without the nucleic acid), as is often needed in ion-counting experiments involving ion-sensitive dyes[10] and buffer exchange.[11] All of the necessary information is derived from measurements on the same sample, thereby extending ion counting into regimes where equilibrium dialysis may not be applicable, such as in the characterization of nucleic acids in difficult-to-dialyze osmolyte solutions.

To demonstrate the feasibility of this approach, we measured SAXS signals at several X-ray energies close to but below the absorption edge of the ions of interest. Previous studies have shown that the number and distribution of monovalent ions in the cloud surrounding DNA and RNA follow the theoretical description given by the nonlinear Poisson−Boltzmann (NLPB) formalism,[7,11,12] providing a benchmark for this measurement. The ASAXS experiment (including setup, background subtraction, fluorescence correction, and solution conditions) has been described previously,[6] except for the SAXS intensity calibration and determination of the anomalous scattering factor. Here we used a well-characterized[7] 25 base pair (bp) DNA duplex. In brief, 0.2 mM duplex DNA was dialyzed extensively in either a 100 mM rubidium acetate (monovalent) or 10 mM strontium acetate (divalent) salt solution with 1 mM Na^+ MOPS buffer (pH 7.0). Control samples in 100 mM sodium acetate were prepared at the same duplex concentrations and buffer conditions. These control samples were used to correct for energy-dependent transmission of all beamline components and to scale the SAXS intensity. We scaled the scattering intensity at the zero angle, $I(0)$, to the square of the number of electrons, n_e^2, measured using water as a SAXS calibrant.[13] Normalized SAXS profiles at different X-ray energies and additional information on using water for the SAXS intensity calibration are given in the Supporting Information.

Near the absorption edge of an ion, the atomic scattering factor (in units of electrons), $f_{ion}(E)$, is given by

$$f_{ion}(E) = f_o + f'(E) + if''(E) \qquad (1)$$

where f_o is the energy-independent solvent-corrected scattering factor (the atomic number Z in vacuum) of the resonant element and E is the X-ray energy. We measured the anomalous scattering factors f' and f'' using the X-ray fluorescence from a buffer solution containing the energy-dependent scatterer (e.g., rubidium acetate) in dilute solution. X-rays were incident on a 1 mm diameter mylar capillary containing this solution; the X-ray fluorescence was collected 90° from the incident beam using an Xflash detector (Rontec, Carlisle, MA). To minimize contributions to the signal from elastic scattering, we placed a KBr foil between the sample and the Xflash detector and used a single-channel analyzer to select the fluorescence signal. CHOOCH,[9] a program commonly used for heavy-atom refinement, was applied to extract f' and f'' from the X-ray fluorescence data (Figure 1). We assumed that f' and f'' of the excess ions near the DNA are identical to those of ions in the bulk solvent. Experimental buffers must be employed for this calibration because the f' values derived from elemental metal foils can deviate from values obtained in dilute solution.

The scattering intensity from the nucleic acid and counterion cloud system, $I(q, E)$, is a function of both the energy E and the momentum transfer q, defined as $q = (4\pi/\lambda) \sin(2\theta/2)$, where λ is the X-ray wavelength and 2θ is the scattering angle. $I(q, E)$ is given by

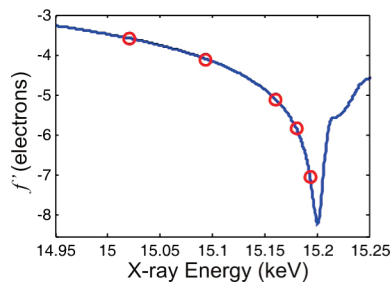$$I(q, E) = |f_{NA}F_{NA} + f_{ion}(E)N_{ions}F_{ion}|^2 \qquad (2)$$

Figure 1. Real part of the anomalous scattering factor for Rb^+ ions determined using CHOOCH as described in the text. The f' values at the energies used in the ASAXS experiment are circled in red. For Sr^{2+} ions, see Figure S1 in the Supporting Information.
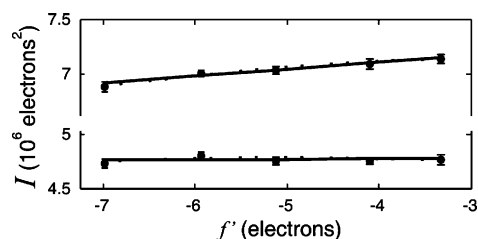

Figure 2. Plots of $I(q, E)$ vs f' using DNA data at $q = 0.07$ Å^{-1}: (top) DNA in Sr^{2+} ions; (bottom) DNA in Na^+ ions (control). Lines show linear (solid) and quadratic (dotted) fits. The linear fit, which neglects the a term in eq 3, provides a correct physical representation of the data.

For measurements carried out below the absorption edge, the f'' term in eq 1 is negligible. The factors F_{ion} and F_{NA} reflect the spatial arrangements of the ion and nucleic acid duplex, respectively (treated as unity at $q = 0$),[12] and f_{NA} describes the effective number of electrons from a nucleic acid duplex. N_{ions} is the number of excess cations present in the ion atmosphere around the nucleic acid. The following simple procedure provides a model-independent method for obtaining N_{ions}. Expansion[6] of eq 2 yields

$$I(q, E) = af'(E)^2 + bf'(E) + c \qquad (3)$$

where

$$a(q) = N_{ions}^2 F_{ion}^2$$

$$b(q) = N_{ions}(2f_{NA}F_{NA}F_{ion} + 2f_o N_{ions}F_{ion}^2)$$

$$c(q) = (f_{NA}F_{NA})^2 + 2f_{NA}f_o N_{ions}F_{NA}F_{ion} + f_o^2 N_{ions}^2 F_{ion}^2$$

The q-dependent functions $a(q)$, $b(q)$, and $c(q)$ are extracted from measurements of $I(q)$ at several different energies by plotting the measured values of $I(f'(E))$ at each q value (or over a small range in q to improve the statistics) and carrying out a quadratic fit. This procedure is repeated for all q, reconstructing the functions of interest point by point.[14] A representative plot of $I(q)$ versus f' at $q = 0.07$ Å^{-1} is shown in Figure 2. Plots of $I(q)$ versus f' at other q values are provided in the Supporting Information. For the system of interest, the contribution to the scattering profile from the nucleic acid was much greater than that from the ion cloud. To assess the relative magnitudes of these terms, we estimated their values at $q = 0$. For a 1 bp DNA molecule with 2 Rb^+ ions, $c(0)/b(0) \approx 50$ and $b(0)/a(0) \approx 200$. The $b(0)/a(0)$ ratio was even larger for Sr^{2+} ions. Therefore, the "a" term was negligible relative to the others and could be ignored, justifying the linear fit $I(q, f'(E)) = b(q)f' +$
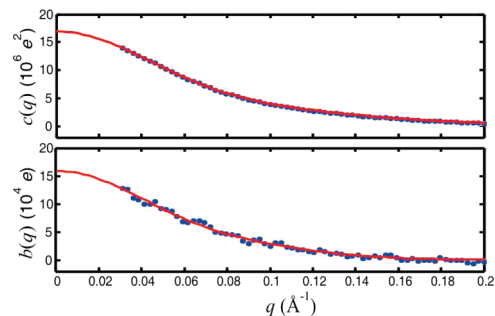

Figure 3. Ion−DNA distribution from ASAXS. DNA scattering dominates in $c(q)$. GNOM fits (lines) allow the extrapolation of $c(q)$ and $b(q)$ to $q = 0$ to find $c(0)$ and $b(0)$ for use in the calculation of N_{ions}.

$c(q)$ (Figure 2). Once the functions $c(q)$ and $b(q)$ have been derived from the data, the number of excess ions is given by

$$N_{ions} = \frac{b(0)}{2\sqrt{c(0)}} \qquad (4)$$

The values of $b(0)$ and $c(0)$ are derived by extrapolating the full $b(q)$ and $c(q)$ curves to $q = 0$ using the program GNOM,[15] which was developed for traditional SAXS analysis. Typical GNOM fits are shown in Figure 3. Using these values in eq 4, we were able to count the number of ions around our 25 bp DNA samples. For monovalent ions, $N_{ions} = 34 \pm 3$, while for divalent ions, $N_{ions} = 19 \pm 2$. We note that NLPB calculations using a finite ion probe radius of 4 Å (effective for monovalent ion atmospheres; see ref 7) predicted the number of excess ions around DNA to be 35.8, in good agreement with our measurements. The NLPB prediction for divalent ions was 21.3 ions around 25 bp DNA. In view of the differences in cation type and DNA length, the number of cations we measured is comparable to the values reported using equilibrium dialysis.[11] However, ASAXS provides both the number of ions and information about their spatial correlation to the nucleic acid, $b(q)$, for comparison to models.[7]

The values of N_{ions} measured here reflect all of the charge-compensating cations around the DNA, i.e., the number of excess ions relative to the surrounding bulk salt solution.[11] Charge neutralization is achieved because negatively charged nucleic acids attract cations and at the same time repel anions from the surrounding solution.[5] The number of excluded anions in this experiment can be inferred because the total charge must be zero.

For SAXS users interested in a relatively quick implementation of this method, the number of ions can also be computed by measuring $I(0)$ at two distinct energies $E_1 < E_2$. From eq 2, neglecting f'', it follows that

$$N_{ions} = \frac{\sqrt{I(0,E_1)} - \sqrt{I(0,E_2)}}{f'(E_1) - f'(E_2)} \qquad (5)$$

This treatment is valuable when only the number and not the spatial distribution of ions is needed. $I(0)$ can be determined using either GNOM or a Guinier approximation. If a CHOOCH measurement and water calibration are unavailable, eq 5 reports a relative number of ions. This can be useful when comparing changes in the ion atmosphere in response to the variation of additional parameters.

In this report, we have demonstrated how ASAXS measurements with SAXS intensity and scattering factor calibrations can simultaneously determine the number and spatial distribution of light counterions around a heavy (more electron dense) polyelectrolyte. The first application of this approach to 25 bp DNA duplexes

yielded ion numbers that are comparable to the NLPB-predicted values. Further applications of this technique will extend ion counting to conditions where important conformational changes in nucleic acids occur, such as RNA folding and protein or ligand binding.

Supporting Information Available: Scattering factors for Sr^{2+} ions, intensity-calibrated SAXS profiles at all energies, notes on using water as a SAXS calibrant, and plots of $I(f'(E))$ versus f' at other q values. This material is available free of charge via the Internet at http://pubs.acs.org.

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