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Counting ions around DNA with ASAXS

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Abstract

The majority of charge compensating ions around nucleic acids forms 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 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 cell¹. 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 like 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 ions⁶. In fact, we recently used ASAXS to highlight differing ion distributions around comparably sequenced double-stranded DNA and RNA helices⁷.

Here, we present an experimentally straightforward extension of ASAXS 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 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 readily obtained 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 dyes¹⁰ and buffer-exchange¹¹. All the necessary information is derived from measurements on the same sample, thereby extending ion-counting into regimes where equilibrium dialysis may not be applicable, for example when characterizing nucleic acids in difficult-to-dialyze osmolyte solutions.

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SUPPORTING INFORMATION: Scattering factors for Sr^{2+} ions, intensity calibrated SAXS profiles at all energies, notes on using water as a SAXS calibrant and $I(f'(E))$ versus f' at other q_0 values. Available free of charge via the Internet at <http://pubs.acs.org>.

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 show 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 set-up, background subtraction, fluorescence correction and solution conditions were described previously⁶ except for the SAXS intensity calibration and anomalous scattering factor determination. Here, we used a well-characterized⁷ 25-base pair DNA duplex. In brief, 0.2 mM of duplex DNA was dialyzed extensively in either a 100 mM RbAcetate (monovalent) or 10 mM SrAcetate (divalent) salt solution with 1 mM Na⁺-MOPS buffer, pH 7.0. Control samples in 100 mM NaAcetate are prepared at the same duplex concentrations and buffer conditions. These control samples are used to correct for energy-dependent transmission of all beamline components and to scale the SAXS intensity. We scale the scattering intensity at the zero-angle, $I(0)$, to the number of electrons squared, n_e^2 , measured using water as a SAXS calibrant¹³. Normalized SAXS profiles at different x-ray energies and additional information on using water for SAXS intensity calibration appear in the supplementary text.

Near an ion's absorption edge, the atomic scattering factor (in units of electrons) is denoted by:

$$f_{ion}(E) = f_o + f'(E) + i f''(E) \quad (1)$$

where f_o is the energy-independent solvent-corrected scattering factor (atomic number Z in vacuum) of the resonant element and E is the x-ray energy. We measure the anomalous scattering factors f' and f'' using x-ray fluorescence from a buffer solution containing the energy-dependent scatterer, e.g. RbAcetate in dilute solution. X-rays were incident on a 1 mm diameter mylar capillary containing this solution; 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 place a KBr foil between the sample and the Xflash detector and use a single channel analyzer to select the fluorescence signal. CHOOCH9, a program commonly used for heavy-atom refinement, was applied to extract f' and f'' from x-ray fluorescence data (Fig. 1). We assume 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 is a function both of energy E and momentum transfer q , ($q = (4\pi/\lambda) \cdot \sin(2\theta/2)$, where λ is the x-ray wavelength and 2θ is the scattering angle) and is given by

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

For measurements carried out below the absorption edge, f'' terms of Eq. 1 are negligible. Terms described by F 's reflect the spatial arrangement of the scattering particles (treated as unity at $q = 0$)¹², and those represented by f_{NA} describe 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} . Expansion⁶ of Eq. 2 yields

$$\begin{aligned}
 I(q, E) &= a \cdot (f'(E))^2 + b \cdot f'(E) + c \quad \text{where} \\
 a(q) &= N_{ions}^2 F_{ion}^2 \\
 b(q) &= N_{ions} \cdot [2f_{NA} F_{NA} F_{ion} + 2f_o N_{ions} F_{ion}^2] \\
 c(q) &= (f_{NA} F_{NA})^2 + 2f_{NA} f_o N_{ions} F_{NA} F_{ion} + f_o^2 N_{ions}^2 F_{ion}^2
 \end{aligned} \tag{3}$$

We extract the q dependent functions, $a(q)$, $b(q)$ and $c(q)$ from measurement of $I(q)$ at several different energies by plotting the measured $I(f'(E))$ at each q value (or over a small range in q to improve statistics), and carrying out a quadratic fit. This procedure is repeated for all q , reconstructing the functions of interest, point by point¹⁴. A representative plot of $I(q_0)$ with respect to f' at $q_0 = 0.07 \text{ \AA}^{-1}$ is shown in Fig. 2. Plots of $I(q)$ versus f' at other q values are provided in the supplementary text. For the system of interest, the contribution to the scattering profile from the nucleic acid is much greater than that from the ion cloud. To assess the relative magnitudes of these terms, we estimate 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 is even larger when considering Sr^{2+} ions. Therefore, the 'a' term is negligible relative to the others and can be ignored, justifying the linear fit $I(q, f'(E)) = b(q) \cdot f' + c(q)$ (Fig. 2). Once the functions $c(q)$ and $b(q)$ have been derived from the data, the number of excess ions is:

$$N_{ions} = b(0) / (2 \sqrt{c(0)}) \tag{4}$$

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 GNOM¹⁵, developed for traditional SAXS analysis. Typical GNOM fits are shown in Fig. 3. Using these values in Eq. 4 we count the number of ions around our 25bp 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 $4 \text{ \AA}$, (effective for monovalent ion atmospheres, see ref. 7) predict the number of excess ions around DNA to be 35.8 in good agreement with our measurements. The NLPB prediction for divalent ions is 21.3 ions around 25bp DNA. Considering differences in cation type and DNA length, the number of cations we measured is comparable to the values reported using equilibrium dialysis¹¹. However, ASAXS provides both the number of ions as well as information about their spatial correlation to the nucleic acid, $b(q)$, for comparison to models⁷.

The N_{ions} measured here reflects all the charge-compensating cations around the DNA i.e. the number of excess ions relative to the surrounding bulk salt solution¹¹. Charge neutralization is achieved because negatively-charged nucleic acids attract cations and, at the same time, repel anions from the surrounding solution⁵. 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

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

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 variation of additional parameters.

In this report, we demonstrate how ASAXS measurements with SAXS intensity and scattering factor calibration 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-base pair DNA duplexes yields 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 like RNA folding and protein or ligand binding.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The real part of the anomalous scattering factor for Rb^+ ions determined using CHOOCH as described in the text. Energies used for ASAXS are shown (circled). For Sr^{2+} ions, see the supporting text.

Figure 2.

$I(q,E)$ vs. f' of DNA data at $q = 0.07 \text{ \AA}^{-1}$. Top curve is DNA in Sr^{2+} ions, bottom curve is DNA in Na^+ ions (control). Lines show linear (solid) and quadratic (dotted) fits. The linear fit, which neglects the a -term provides the correct physical representation of the data.

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