The International Journal of Biochemistry & Cell Biology 45 (2013) 429–437

Contents lists available at SciVerse ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Review

Applications of small-angle X-ray scattering to biomacromolecular solutions

Maxim V. Petoukhov, Dmitri I. Svergun*

European Molecular Biology Laboratory, Hamburg Outstation, Notkestrasse 85, 22607 Hamburg, Germany

ARTICLE INFO

Article history:
Received 24 August 2012
Received in revised form 25 October 2012
Accepted 30 October 2012
Available online 9 November 2012

Keywords:
Solution scattering
Data analysis
Low resolution structure
Biological macromolecules
Hybrid modeling

ABSTRACT

Small-angle scattering of X-rays (SAXS) is an established method for low-resolution structural characterization of biological macromolecules in solution. Being complementary to the high resolution methods (X-ray crystallography and NMR), SAXS is often used in combination with them. The technique provides overall three-dimensional structures using *ab initio* reconstructions and hybrid modeling, and allows one to quantitatively characterize equilibrium mixtures as well as flexible systems. Recent progress in SAXS instrumentation, most notably, high brilliance synchrotron sources, has paved the way for high throughput automated SAXS studies allowing screening of external conditions (pH, temperature, ligand binding *etc.*). The modern approaches for SAXS data analysis are presented in this review including rapid characterization of macromolecular solutions in terms of low-resolution shapes, validation of high-resolution models in close-to-native conditions, quaternary structure analysis of complexes and quantitative description of the oligomeric composition in mixtures. Practical aspects of SAXS as a standalone tool and its combinations with other structural, biophysical or bioinformatics methods are reviewed. The capabilities of the technique are illustrated by a selection of recent applications for the studies of biological molecules. Future perspectives on SAXS and its potential impact to structural molecular biology are discussed.

Contents

1. Introduction 429
2. *Ab initio* shape reconstruction 430
3. Validation and screening of concurrent high-resolution models 431
4. Rigid body modeling of complex particles and multidomain proteins 431
5. Analysis of polydisperse systems 432
6. Examples of SAXS applications 432
7. Validation of an unusual oligomeric assembly in the crystal 432
8. Study of modular protein structure by joint use of SAXS and NMR 433
9. Rigid body analysis of calmodulin-activated glutamate decarboxylase 434
10. Study of ionic strength dependence of actin complexes 434
11. Future prospects: high throughput, full automation 435
12. Conclusions 435
Acknowledgement 435
References 435

1. Introduction

The field of modern structural biology is dominated by a systematic approach in which the focus is set on the structural studies of complex objects, molecular machines and processes (Gavin et al., 2006; Abrahams et al., 2011). By structurally analyzing the interactions within biological systems as well as predicting their behavior based on the composition, the relationship between structural mechanisms and functionality can be elucidated. For this, hybrid methods are often applied combining various bioinformatics and

Abbreviations: 1D, one-dimensional; 3D, three-dimensional; βT, β-thymosin; CaM, calmodulin; CH3, chimera 3; CibD1, isolated first β-thymosin domain of *Drosophila* Ciboulot; D_{max}, maximum size; DR, dummy residue; E2, dihydrolipoyl acyl-transferase; EM, electron microscopy; EOM, ensemble optimization method; FRET, fluorescence resonance energy transfer; Gad, glutamate decarboxylase; HPLC, high-performance liquid chromatography; I_0, forward scattering intensity; MO, maximum occurrence; NMR, nuclear magnetic resonance; PCS, pseudocontact shifts; RDC, residual dipolar coupling; R_g, radius of gyration; SAXS, small-angle X-ray scattering; *V*, volume; WAXS, wide-angle X-ray scattering.

* Corresponding author. Tel.: +49 4089902125; fax: +49 4089902149.
E-mail address: Svergun@EMBL-Hamburg.DE (D.I. Svergun).

1357-2725/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biocel.2012.10.017

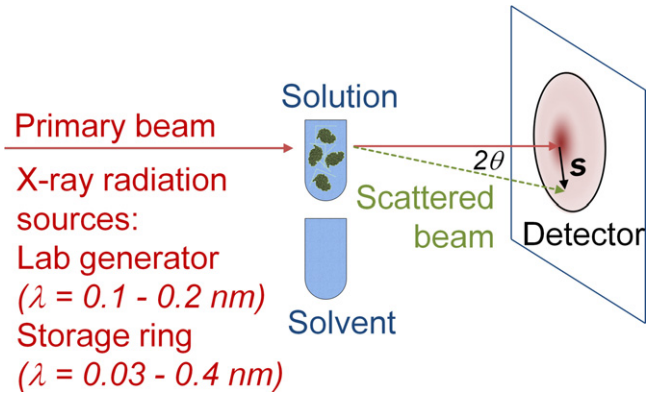

Fig. 1. Schematic representation of a solution SAXS experiment.

experimental techniques. They cover a broad range of temporal and spatial resolution and thus, provide a quantitative understanding of the entire system.

Small-angle X-ray scattering (SAXS) is a universal structure probe for a wide variety of non-crystalline objects (*e.g.* macromolecular solutions, detergents, nanocomposites, alloys, synthetic and bio-polymers, biomaterials, organic/inorganic films *etc.*). SAXS enables structural studies in a broad range of molecular sizes from kDa to GDa and in nearly native to extreme experimental environments (*e.g.* high pressure or cryo-frozen). This allows one to simultaneously monitor the interactions in hierarchical systems at different levels of structural organization. SAXS is also able to operate *in situ* and in a time-resolved manner yielding unique information about the kinetics and dynamics of processes.

In a typical biological SAXS experiment, a monochromatic and collimated X-ray beam illuminates macromolecules which are oriented chaotically in solution and their scattering pattern is recorded by an X-ray detector. For dilute solutions, the scattered intensity I is isotropic and depends only on the scattering angle 2θ (Fig. 1). Measurements of the sample containing the macromolecules are complemented by those of the blank solvent (typically dialysis buffer) and the latter scattering is afterwards subtracted from the measured sample intensities. The resulting scattering profile is usually expressed as a function of the momentum transfer $s = 4\pi \sin\theta/\lambda$ (here, λ is the wavelength) and is proportional to the scattering from a single particle averaged over all orientations as well as to the solute concentration.

A scattering profile from monodisperse solution of non-interacting identical particles carries information about the major geometrical parameters of the particle. Particularly, molecular mass (*MM*) of the solute and its radius of gyration (R_g) are derived from the slope of the Guinier plot $\ln(I(s))$ *versus* s^2 (which is linear at low s) (Guinier and Fournet, 1955). In addition, the values of the hydrated particle volume (V) and its specific surface (S) can be obtained using the so-called Porod invariant (Porod, 1982). *MM* and *V* provide estimates of the possible oligomeric state or, in case of complexes, may give a hint on the (partial) dissociation level. Here, MM is calculated by comparison of the forward scattering of a reference sample (*i.e.* standard protein), which requires precise information about the solute concentration. The use of volume, although somewhat less accurate, does not depend on concentration (Petoukhov et al., 2012). The maximum distance within the particle (D_{max}) is also readily available from the Fourier transformed SAXS pattern (Svergun, 1992). The folding state of the particle can be assessed utilizing the Kratky plot ($I(s)s^2$ *versus* s): a bell-shaped profile is typical for globular proteins whereby a monotonously increasing function points to unfolded ones (Mertens and Svergun, 2010).

In addition, to the overall parameters, one-dimensional (1D) solution scattering profiles $I(s)$ allow one to meaningfully analyze three dimensional (3D) structures (Fig. 2). First of all, low resolution particle shapes can be reconstructed *ab initio* (*i.e.* without any prior information) from the scattering patterns. For the cases in which a high resolution model is available, it can be validated against the experimental SAXS data and possible conformations or oligomeric states can be rapidly screened to identify the biologically relevant structure. In case of mixtures and flexible systems, solution scattering provides means for quantitative description of the sample composition. For multisubunit particles, the quaternary structure of the complex can be reconstructed based on the models of individual subunits by applying rigid body analysis. The major SAXS-based modeling methods and some applications will are presented below.

2. *Ab initio* shape reconstruction

The reconstruction of a 3D model from 1D scattering pattern is an ill-posed problem as it potentially yields multiple shapes compatible with one and the same scattering profile. Still, the scattering intensity can be considered to be a particle shape's footprint and the ambiguity of the reconstruction can be reduced by imposing restraints to the model. These restrictions, in the case of *ab initio* shape determination, consist in representing the macromolecule as a homogeneous and compact particle at low resolution. Such a concept is implemented in an approach representing the particle as an ensemble of (several thousands of) densely packed beads on a regular grid (Chacon et al., 1998; Svergun, 1999). Each of these beads belongs either to the particle (index = 1) or to the solvent (index = 0) so that the shape is described by a binary configuration vector. A widely used bead modeling algorithm implemented in the programs DAMMIN (Svergun, 1999) and DAMMIF (Franke and Svergun, 2009) applies simulated annealing (SA) to build a compact interconnected configuration of beads that fits the experimental data by minimizing discrepancy between the experimental data $I_{exp}(s)$ and a scattering profile computed from the model $I_{calc}(s)$:

$$\chi^2 = \frac{1}{N-1}\sum_{j=1}^{N}\left[\frac{I_{exp}(s_j) - cI_{calc}(s_j)}{\sigma(s_j)}\right]^2 \quad (1)$$

where c is a scaling factor, N is the number of points and σ denotes the experimental errors. The search can be done either in a confined volume (DAMMIN) or on an unlimited grid (DAMMIF). In the former case, the search volume can be defined by the maximum size of the particle D_{max} which is either deduced from the SAXS data or is taken from complementary structural methods *e.g.* electron microscopy maps. In the latter case, the available space is expanded as necessary in the course of modeling.

For oligomeric particles consisting of identical subunits, symmetry can be taken into account as a rigid constraint so that the symmetrically related beads are added/removed to the model simultaneously. For multicomponent particles (*e.g.* nucleo- and lipo-proteins or protein complexes) there is a possibility to extend the approach to a multiphase bead modeling where the phase index varies up to the number of components describing not only the overall shape but also the internal organization of the composite molecule. The prerequisite for this, however, is the availability of the scattering profiles from the individual components (or their combinations) so that they are fitted simultaneously with the data of the intact particle (Svergun and Nierhaus, 2000).

Ab initio modeling is now routinely used in various SAXS-based studies providing a very useful first glance onto the molecule at a low resolution (about 1.5–2 nm). Moreover, the bead models can be utilized in the complementary techniques, *e.g.* as starting points for image classification in electron microscopy.