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Review article

Using Lamm-Equation modeling of sedimentation velocity data to determine the kinetic and thermodynamic properties of macromolecular interactions

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ABSTRACT

The interaction of macromolecules with themselves and with other macromolecules is fundamental to the functioning of living systems. Recent advances in the analysis of sedimentation velocity (SV) data obtained by analytical ultracentrifugation allow the experimenter to determine important features of such interactions, including the equilibrium association constant and information about the kinetic off-rate of the interaction. The determination of these parameters is made possible by the ability of modern software to fit numerical solutions of the Lamm Equation with kinetic considerations directly to SV data. Herein, the SV analytical advances implemented in the software package SEDPHAT are summarized. Detailed analyses of SV data using these strategies are presented. Finally, a few highlights of recent literature reports that feature this type of SV data analysis are surveyed.

1. Introduction

Macromolecular interactions lie at the heart of modern molecular biology. Proteins can interact with themselves (a homo-association) or with other molecules (a hetero-association), like small metabolites, other proteins, nucleic acids, and carbohydrates. In studying these interactions *in vitro*, one of the most relevant quantities for biochemists to determine is strength of the interaction, expressed as the equilibrium association constant (K_A). Commonly, this quantity is reported as the dissociation constant, or K_d ($K_d = 1/K_A$). Numerous means have been devised to measure this quantity, including isothermal titration calorimetry, fluorescence quenching, fluorescence anisotropy, electrophoretic mobility, equilibrium dialysis, liquid chromatography, and others (e.g. [1,2]).

Analytical ultracentrifugation (AUC) is emerging as a potent means to study the interactions of macromolecules [1,3–7]. Sedimentation equilibrium (SE) has long been used to determine the values of K_A for homo- and hetero-associations [1,8]. In this method, the samples are centrifuged at speeds that generate shallow concentration gradients. These gradients are stable once all species have achieved physical and thermodynamic equilibrium. The shape of the gradients contains information regarding the masses of all species present, and experiments performed at several component concentrations and rotor speeds may be analyzed to yield complex masses and dissociation constants. Although several disadvantages to SE are known, recent advances in data analysis have overcome some of them [9,10]. The technique remains a valuable tool for the study of macromolecular interactions, but its main disadvantage lies in the time necessary to complete the experiment. The most data-rich SE method, called "long-column" SE, may take as long as a week to complete, with the concomitant demand that the sample remains stable during this time. "Short-column" SE may take only hours to complete [11], but also has a significantly smaller data basis.

By contrast, the sedimentation velocity (SV) configuration of analytical ultracentrifugation takes only hours and is comparatively data rich. In the work that follows, new advances in the direct modeling of SV data with numerical solutions to the Lamm Equation coupled to reaction fluxes are summarized. To introduce new practitioners to how these approaches can be used in SEDPHAT, detailed analyses are presented: one for a homo-association, the other characterizing a hetero-association. Finally, some recent results using this approach are discussed.

Abbreviations: Arp2/3, actin related protein 2–actin related protein 3 complex; AUC, analytical ultracentrifugation; LE, Lamm Equation; LEq, Lamm Equation coupled to kinetic reaction fluxes *q*; MSSV, multisignal sedimentation velocity; OD, optical density; SE, sedimentation equilibrium; SV, sedimentation velocity analytical ultracentrifugation; ML, Marquardt–Levenberg; VCA, verprolin homology–central region–acidic region; PAS, PER-Arnt-Sim; PAS-A, the N-terminal PAS domain of KinA; EGTA, ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid; MLL-1, mixed-lineage leukemia protein-1; WDR5, WD repeat protein 5; RbBP5, retinoblastoma-binding protein-5; Ash2L, absent small homeotic-2-like protein; r.m.s.d., root-mean-square deviation; S(1), the sedimentation coefficient of a monomer; S(2), the sedimentation coefficient of a dimer; sAB, the sedimentation coefficient of the AB complex; TODA, time of data acquisition.

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2. Theory

2.1. Background

SV is very useful for studying non-interacting solutes, and it may also be used for the study of macromolecular interactions. This methodology features higher rotor speeds than SE. The macromolecular solutes thus migrate through the solution column and become localized very close to the centrifugal portion ("bottom") of the centrifugation cell. The concentration gradients formed are monitored during the entire course of the experiment (Fig. 1A). A sedimenting macromolecule will give rise to a sigmoid concentration profile; roughly speaking, the inflection point of this feature is called a "boundary." The shape and velocity of the boundaries contains information regarding the size and shape of the sedimenting particle. As centrifugal force moves the boundary centrifugally, diffusion acts to make the boundaries progressively shallow during the course of the experiment (i.e. they become "diffusionally broadened"). The presence of two particles of sufficiently divergent size results in two boundaries (Fig. 1B), and so on.

The partial differential equation that describes the evolution of the boundaries was first formulated by Lamm in 1929 [12]. It is thus called the "Lamm Equation" (LE), and may be formulated thus for an ideal particle sedimenting in a sector-shaped centrifugal cell:

$$\frac{\partial \chi}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left[rD\frac{\partial \chi}{\partial r} - s\omega^2 r^2 \chi\right], \tag{1}$$

where χ, D, and s are the concentration, diffusion coefficient, and sedimentation coefficient, respectively, of the particle, t is time, r is the radius from the center of rotation, and ω is the angular velocity of the rotor. The LE describes the ideal transport processes occurring during the course of the SV experiment, including sedimentation, diffusion, and even floatation. There are no known exact analytical solutions to the LE, but there are many approximate analytical solutions that consider the cases of no diffusion, rectangular cell geometry, etc. [13]. However, with the advent of inexpensive, powerful computers, numerical solutions to the LE are now readily available and routinely used [8].

The implications of the accessibility of quickly calculated numerical solutions to the LE are manifold. The solutions allow SV data obtained from ideal, non-interacting macromolecules to be directly modeled. Several software programs are available for this purpose, including LAMM, SEDANAL, ULTRASCAN, SEDFIT, and SEDPHAT [6,14–17]. In addition, the ease of this computation facilitates the direct description of the boundaries as a continuous distribution that scales a large number ($\geqslant 50$) of LE solutions. This approach was first described by Schuck [18], and the mathematical formalism is:

$$a(r,t) \cong \int_{s\ \min}^{s\ \max} c(s)\chi(s, D(s), r, t)ds, \tag{2}$$

where $a(r,t)$ is the signal measured by the centrifuge, χ is a concentration profile that represents an LE solution of a non-interacting species as a function of the parameters listed, and $D(s)$ is the diffusion coefficient calculated as a function of s and with the assumption of all species having the same frictional ratio (f_r).

Although the scheme of directly fitting LE solutions to SV data of proteins and other biologic macromolecules can work well, the LE as formulated above (Eq. (1)) does not account for chemical reactions, i.e. the interaction of multiple species present in the centrifugal cell. Thus, adjustments must be made to the LE so that it may be used to model SV data in which such interactions are known to occur. Examples of macromolecular interactions are abundant in biology. Proteins interact with themselves to form oligomers (a homo-association) and with other proteins, forming complexes (hetero-associations). In order to use the LE to analyze such interactions in the SV setting, the LE must be combined with reaction fluxes and information on the equilibrium association constant (K_A). For an instantaneously equilibrating system, the data may be treated using a weighted average for s and a gradient average for D [19]. For hetero-associations, explicit reaction fluxes, q_k, may be considered in an equation system:

$$\begin{aligned} &\frac{\partial \chi_k}{\partial t} + \frac{1}{r}\frac{\partial (rJ_{k,tr})}{\partial r} = q_k \\ &J_{k,tr} = s_k\omega^2 r\chi_k - D_k\frac{\partial \chi_k}{\partial r}, \end{aligned} \tag{3}$$

where χ_k is the concentration of the component k, D_k and s_k are its diffusion and sedimentation coefficient, respectively, q_k is the local reaction rate, and $J_{k,tr}$ is component's transport flux [13]. The fluxes are dependent on the component and complex concentrations, which are dependent on K_A. Both of these approaches are implemented in the freeware program SEDPHAT [5,19]. Hereafter, this type of analysis is referred to as "LEq," for "Lamm Equation coupled to reaction fluxes q."

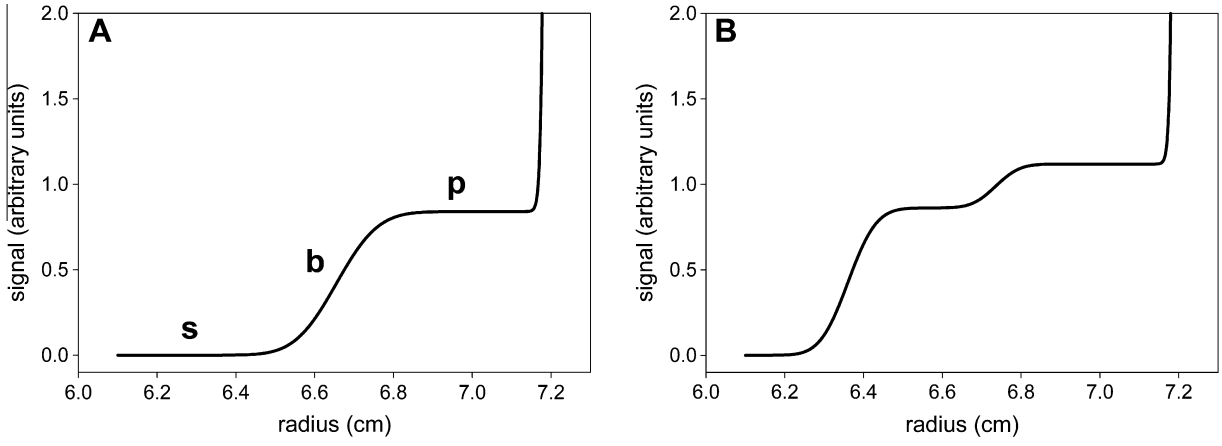

Fig. 1. Boundaries in sedimentation velocity experiments. (A) A typical sedimentation velocity concentration profile. The "top" of the centrifugation cell is at the left of the figure, and the "bottom" is at the right. The boundary, plateau, and solvent region are marked with a "b," "p," and "s," respectively. Note the area of solute buildup near to the bottom of the cell. (B) A concentration profile with two boundaries.