Complex of Two Proteins in the Presence of a small Molecule

Abstract:

Eight samples were received, containing various mixtures of protein A, protein B, molecule 1, and molecule 2 for biophysical characterization at the Canadian Center for Hydrodynamics. The objective of this experiment was to analyze each of the samples using analytical ultracentrifugation (AUC) and to assess if a ternary complex is formed when an equimolar mixture of protein A and B is combined with either molecule 1 or 2.

Methods:

UV-visible absorbance (220 – 700 nm) was measured for each sample at room temperature (22 °C) with a ThermoFisher Genesys 50S benchtop spectrophotometer in a 1 cm pathlength quartz cuvette (Figure 1). Absorbance values at 280 nm of all samples varied from 0.519 to 0.926 OD for all samples, suggesting inconsistent concentrations across all samples, and the possibility of background absorbance resulting from buffer components (Table 1). The absorbance of the complex mixtures at 280 nm was determined to be ouside of the dynamic range of the AUC's detector, resulting in an OD of higher than 0.9 OD in the AUC due to the 1.2 cm pathlength of the AUC cell, therefore, samples containing proteins A and B with molecule 1 or 2 were measured at 296 nm (Table 1). The actualmolar concentrations of the stock solutions for proteins A and B were calculated using the measured absorbance of the sedimenting components at 280 nm, subtracting any baseline absorbances, and using the molar extinction coefficients generated in UltraScan-III for proteins A and B. With the measured absorbance at 280 nm from the AUC and the extinction coefficients generated in UltraScan-III, the molar stock concentrations of protein A and B were determined to be 6.21 and 4.67 μM, respectively (Table 2). These measurements suggest that due to the potential mismatch in molar ratio for the mixtures, it is likely that non-equimolar mixtures were examined.

Table 1: Measurements recorded on the Genesys 50S for AUC sample prep

Sample	Absorbance at 280 nm	Absorbance at 296 nm
Protein A	0.566 OD	0.297 OD
Protein B	0.547 OD	0.207 OD
Protein A + Molecule 1 (1:1)	0.596 OD	0.467 OD
Protein B + Molecule 1 (1:1)	0.748 OD	0.450 OD
Protein A + Molecule 2 (1:1)	0.835 OD	0.479 OD
Protein B + Molecule 2 (1:1)	0.519 OD	0.319 OD
Protein A + Protein B + Molecule 1 (1:1:1)	0.906 OD	0.506 OD
Protein A + Protein B + Molecule 2 (1:1:1)	0.926 OD	0.493 OD

Table 2: Measurements recorded on the AUC using the 2DSA-IT analysis

Protein	Absorbance	Concentration
A	0.273 OD	6.209 µmol
B	0.397 OD	4.666 µmol

AUC Measurements:

All samples were spun in cells assembled with 2-channel epon centerpieces and quartz windows, and run in an An50Ti rotor. The samples were run using a Beckman-Coulter Optima AUC instrument at a speed of 50 krpm for 10 h at 21 °C. Experimental data were collected in intensity mode at 280 and 296 nm to optimize signal-to-noise ratio and signal linearity.

Data Analysis:

All samples were analyzed with UltraScan-III using sedimentation velocity analytical ultracentrifugation (SV AUC) [1]. Data analysis was performed using two-dimensional spectrum analysis (2DSA), removing time- and radially-invariant systematic noise components, and fitting the meniscus position [2]. A final refinement included an iterative 2DSA for each dataset and was followed by a Monte Carlo analysis (2DSA-MC) [3] and a parametrically constrained spectrum analysis (PCSA) [4]. A van Holde and Weischet analysis was used to determine diffusion-corrected integral sedimentation coefficient distributions [5].

Results:

All results, including overlays of experimental data with fitted models from the 2DSA-MC analysis, are available in the LIMS report section for review. The fits provided excellent RMSD values and low residuals, suggesting a high confidence in the results.

Figure 1 shows the diffusion corrected integral sedimentation coefficient distributions from the van Holde Weischet analysis. This plot shows that all samples exhibit similar sedimentation coefficients around 3 S. Protein A and all mixtures containing protein A show the presence of aggregation, while protein B gives rise to homogeneous distributions, suggesting high purity. The presence of the molecules 1 and 2 has no visible effect on the sedimentation profiles of proteins A or B, or their mixtures, suggesting that there is no complex formed.

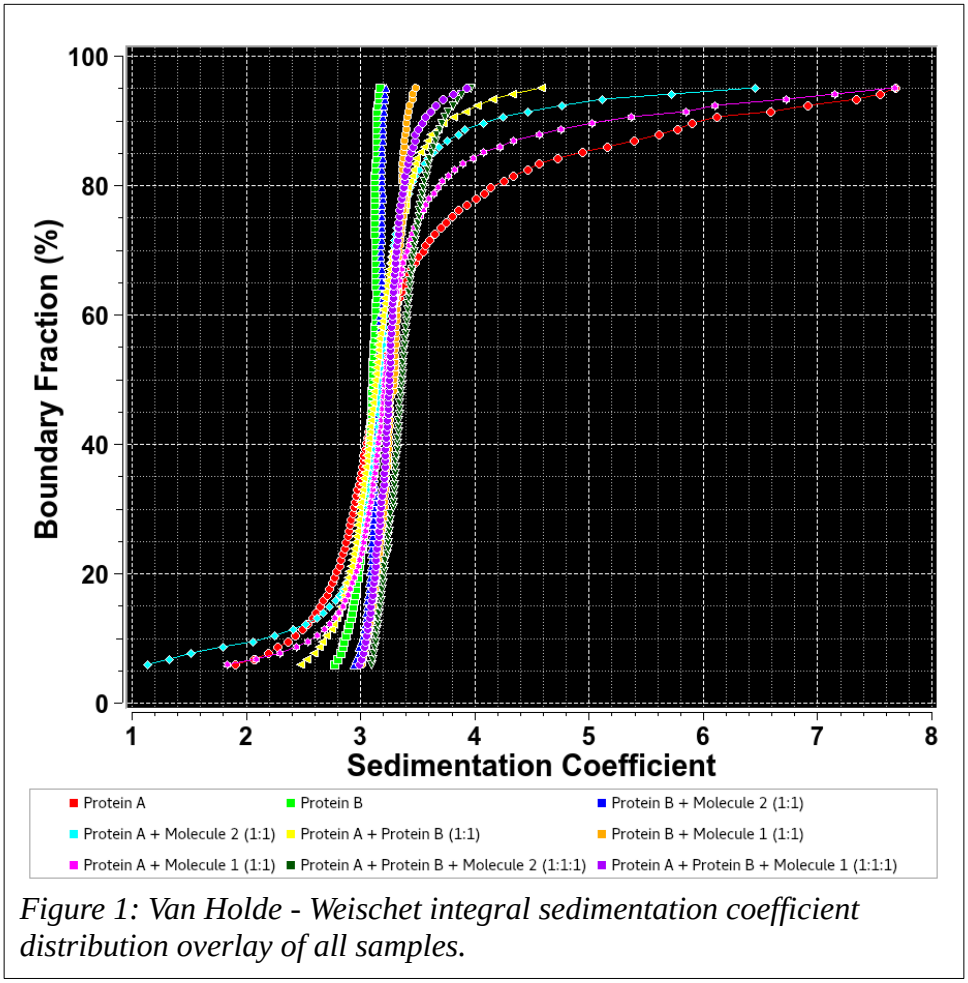

Figure 1: Van Holde - Weischet integral sedimentation coefficient distribution overlay of all samples.

Discussion:

The observations did not agree with the hypothesis of a ternary complex forming of proteins A and B in the presence of either molecule 1 or 2. The preparation of protein A suggests the presence of aggregates and other impurities, and the unequal concentrations of the two proteins suggests that the anticipated complex formation is potentially compromised. Further studies should be carried out with multi-wavelength AUC to distinguish interactions by following unique chromophores and extinction profiles of each component in any given mixture [6], and careful UV spectroscopy should be used to prepare mixtures that are properly matched in their molar ratios.

References:

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