APPLICATION NOTE

AN1610: Stoichiometry of intrinsically disordered protein complexes

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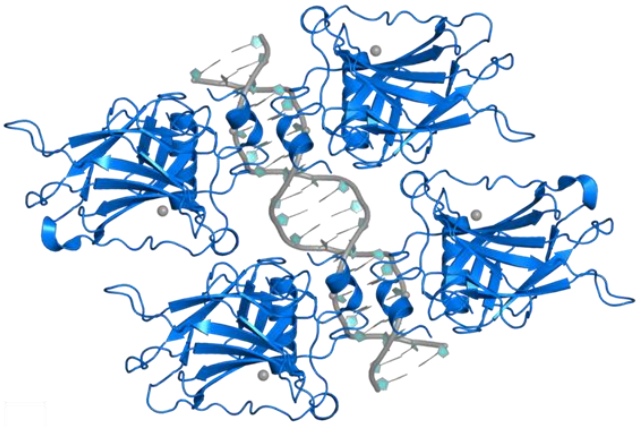
Introduction

The S100 family is a group of dimeric, calcium-binding proteins which have been found to be overexpressed in several types of cancer. S100 proteins bind to the C-terminal region (293-393) of p53, the most critical tumor suppressor involved in apoptosis, cell cycle arrest and DNA repair. Since S100 is dimeric and the C-terminus of p53 encompasses a tetramerization domain, the potential range of complexes incorporating p53 and S100 is large.



p53 tetramer interacting with DNA. Source: PDBe 3kz8

The p53 variants have a large fraction of intrinsically disordered structure and do not elute according to globular protein standards in size-exclusion chromatography (SEC). However, multi-angle light scattering determines molecular mass from first principles and does not depend on SEC column calibration standards. Combining SEC with static multi-angle light scattering (SEC-MALS) provides an ideal system to study the complex formation of S100 proteins and p53; it determines the molecular weight of the complexes independently of protein standards and retention time, under native buffer conditions.

We utilized SEC-MALS to investigate how S100 proteins bind to p53 in its different oligomeric states, using monomeric (L344P) and dimeric (L344A) p53 mutants.

Materials and Methods

The experimental system consisted of an HPLC and SEC column plumbed in series with a DAWN® MALS detector and an Optilab® differential refractive index (dRI) detector. Data collection and analysis were performed in the ASTRA® software. The combination of MALS and dRI concentration data suffices to calculate the solution's weight-average molar mass at each elution volume in the chromatogram, as plotted in the figures.

The p53 mutants and S100 proteins were first characterized separately by SEC-MALS to determine their molecular masses and homogeneity. They were then incubated at various stoichiometric ratios—4:1, 2:1, 1:1, 1:2 and 1:4—and analyzed on the SEC-MALS system.

Results and Discussion

Molar mass results of the individual p53 proteins are overlaid with the light scattering (thin solid lines) and dRI chromatograms (dotted lines) in Figure 1. The molar masses correspond to a monomer of 11.2 kDa for the L344P variant, a dimer for the L344A variant and a tetramer for p53 wild-type. Slight curvature of the wild type and L344A mutant molar mass plots correlates with concentration within the peak, indicating a small degree of dynamic equilibrium at these concentrations. The monomeric L344P mutant is quite homogeneous across its peak.

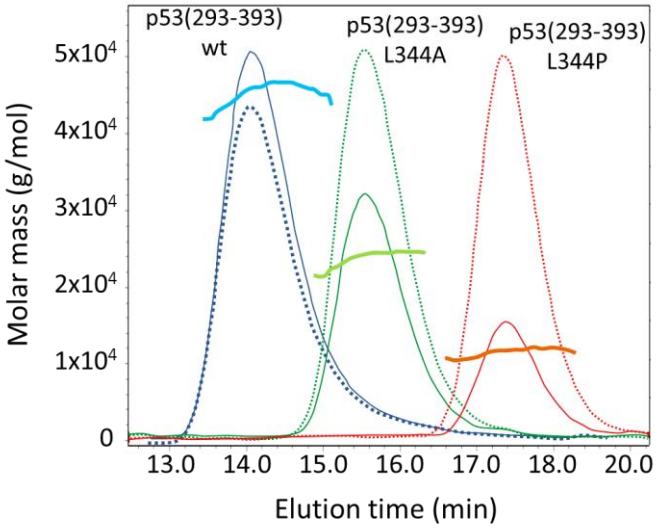


Figure 1. Analytical SEC-MALS of p53(293-393) variants. Thin, solid lines are light scattering chromatograms, dotted lines the corresponding dRI signals and thick solid lines are the calculated molar masses.

Light scattering chromatograms and calculated molar masses for the pre-incubated mixtures are presented in Figure 2. Monomeric p53 (293-393) L344P (11.2 kDa) and dimeric S100B (21.4 kDa) both eluted with a retention time of ~17.5 min. The complex molar mass of ~32 kDa at ~16 min. is independent of the relative ratio of the two proteins, indicating that one dimer of S100B binds only one monomer of p53. Again, concentration-dependent curvature of the molar mass in the complex peak indicates rapid equilibration of complexes and monomers during the elution, a property not evident in standard SEC.

	S100B	w.t.	L344A	L344P
Native oligomer:	dimer	tetramer	dimer	monomer
Stoichiometry	Complex forms with S100B?			
1:1		-	-	-
2:1		-	√	√
2:2		-	-	-
4:1		-	-	-
8:4		√	-	-

Table 1. Summary of oligomers and protein-protein complexes found. Stoichiometry refers to the number of each monomer in the complex.

In additional data not shown here, it was found that no complexes consisting of a dimer of S100B and two monomers of w.t. p53 (~43 kDa), or of two dimers binding one monomer of p53 (~54 kDa), could be detected. However, S100B was able to disrupt the dimeric p53 (293-393) L344A mutant and form a complex with the monomer. Interestingly, SEC-MALS revealed that S100B was also able to form a stable complex with tetrameric w.t. p53 (293-393); the molar mass of the complex consisted of 4 dimers of S100B bound to a tetramer of p53.

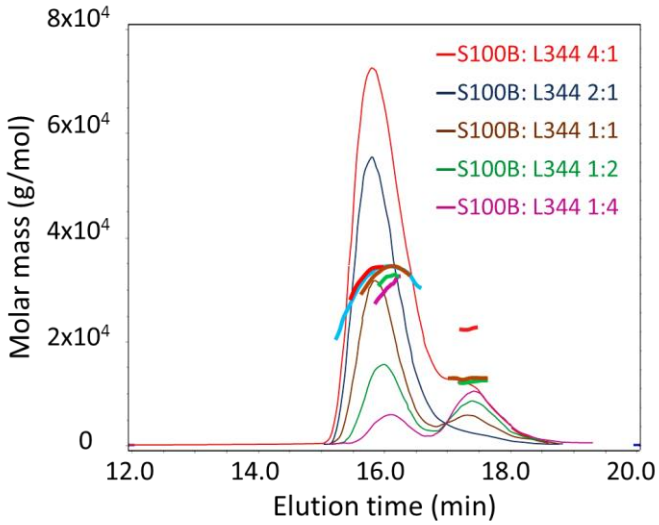


Figure 2. SEC-MALS results of S100B and the mutant p53(293-393) L344P, pre-incubated in different stoichiometric ratios: light scattering chromatograms and calculated molar masses across each peak. Curvature of the molar masses at the 16-minute peak indicates dynamic equilibrium of the complex.

Conclusions

SEC-MALS helped understand how S100 proteins influence the oligomerization of p53. Based on these results and in vivo studies, we established a binding model where S100 proteins can activate tetrameric p53 but inhibit p53 activity binding to the monomer by shifting the tetramerization equilibrium. Hence, SEC-MALS is a valuable tool in structural biology for studying the stoichiometry and formation of many types of protein-protein complexes.