

BACKGROUND

This experiment represents the initial part of a long project that aims at elucidating the binding mode and the structure of the Prep1-Pbx1-HoxB1-DNA complex. Indeed, the protein Prep1 in complex with its binding partner Pbx1 and possibly also with HoxB1 acts as tumor suppressor by interacting with DNA. In parallel with X-ray crystallography studies that, once obtained the crystals, will elucidate the structure of the complex, SAXS is used to clarify the Prep1/Pbx1 interactions and the DNA binding architecture in solution.

RESULTS OF PRELIMINARY WORK

SAXS datasets of the binary (Prep1-Pbx1 ~ 73 kDa) and the ternary (Prep1-Pbx1-DNA ~ 90 kDa) complexes have been collected at ESRF (ID14-3). Both the complexes with and without DNA appear to be soluble, homogeneous and suitable for SAXS. In addition from my SAXS data it seems that Prep1-Pbx1 complex undergoes some shape rearrangement upon binding DNA. Due to the conformational change after DNA binding, I did not obtain a satisfactory model from MONSA multiphase reconstruction. Although the quality of my SAXS data allows me to obtain *ab initio* models of the two complexes, due to the limitation of SAXS method, I am not able to distinguish in my models the DNA from the protein.

RESULTS

SAXS datasets of the binary (Prep1-Pbx1 ~ 75 kDa) and the ternary (Prep1-Pbx1-DNA ~ 87 kDa) complexes have been already collected at ESRF (ID14-3). Both the complexes with and without DNA are soluble, monodisperse and suitable for SAXS. Although Prep1-Pbx1 binary complex at higher concentrations tends to aggregate, data can be collected free from interparticle effects from low concentrations (approx 0.5 mg/ml) estimated from the change in R_g , D_{max} , and Volume (table 1).

protein	concentration	R_g (nm)	D_{max} (nm)	Volume (nm ³)	mW (kDa)	expected mW (kDa)
Prep1Pbx1	7 mg/ml	5,77	19,60	311	194	75
	3.5 mg/ml	4,88	17,01	203	127	75
	0.6 mg/ml	4,23	14,30	174	109	75
Prep1Pbx1 + DNA	14 mg/ml	4,30	15,25	162	101	87
	7 mg/ml	4,30	15,24	160	100	87
	3.5 mg/ml	4,30	15,00	158	98	87
	1.8 mg/ml	4,30	15,00	155	97	87

Table 1: Summary of SAXS parameters calculated with Primus and Gnom (ATSAS 2.4 package). The SAXS data provided *ab initio* models (figure 1) of the two complexes and although they overlay it is not possible to adequately distinguish the position of the DNA relative to the protein as the reconstruction from Prep1-Pbx1-DNA complex is affected by the density differences between protein and DNA. Multiphase reconstruction from this data was also inconclusive probably due to conformational changes upon DNA binding.