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# The solution structure of apo-iron regulatory protein 1

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ABSTRACT

Iron is a cofactor for many proteins that are involved in essential metabolic processes. However, iron must be strictly regulated because it can react with oxygen to generate cytotoxic reactive oxygen intermediates. Iron regulatory protein 1 (IRP1) is a bi-functional protein that can act either as a post-transcriptional regulator of mRNAs containing iron responsive elements, or as a [4Fe–4S] cluster-containing cytosolic aconitase. Previous X-ray crystallography results show that IRP1 is in an open L-shape conformation when bound to IRE-RNAs, and in a globular conformation when it binds an iron–sulfur cluster. The structure of apo-IRP1 and the mechanism by which it transforms to either functional state is unknown. Therefore, small angle X-ray scattering was used to determine the low resolution solution structure of apo-IRP1 and to characterize its biophysical properties. These results show that apo-IRP1 has a radius of gyration ($R_g$) of 33.6 ± 0.3 Å, and a $D_{max}$ of 118 ± 2 Å. The *ab initio* and rigid-body modeling results show that apo-IRP1 is in an open conformation in solution, and the ensemble optimization results show that the molecules stay narrowly distributed about a $R_g$ of 33–34 Å. The open apo-IRP1 conformation seems optimal for subsequent conversion to either functional end state: RNA-binding, or cytosolic aconitase.

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## 1. Introduction

Iron is an essential element utilized by most organisms. However, iron levels must be strictly regulated in order to prevent deleterious effects of iron insufficiency or iron overload. In metazoans, iron regulatory protein 1 (IRP1) is a bi-functional protein that maintains iron homeostasis by integrating post-transcriptional regulation of iron metabolism genes with the cellular iron status (Anderson et al., 2012). Elucidations of the crystal structures of IRP1 in complex with iron responsive element (IRE) RNA (Walden et al., 2006) and in the form of cytosolic (c-) aconitase (Dupuy et al., 2006) were major steps forward in determining the structural principles responsible for IRP1 bi-functionality. Although much progress has been made in understanding the two functional roles of IRP1, little is known about the structure of the apo-protein and how it acquires either the IRE-RNA or the [4Fe–4S] cluster.

Prior to this work, apo-IRP1 was characterized by several biochemical and biophysical methods. Proteolysis studies showed that apo-IRP1 was more susceptible to degradation than c-aconitase (Schalinske et al., 1997). Neutron scattering experiments were the first to determine the radii of gyration ($R_g$s) of the different states of IRP1, and demonstrate that apo-IRP1 has a $R_g$ larger than the protein when complexed with IRE-RNA (Brazzolotto et al., 2002). Analytical ultracentrifugation results were consistent with the neutron scattering findings, showing a decrease in the hydrodynamic (Stokes) radius of IRP1 when binding IRE (Yikilmaz et al., 2005). Together, these experiments suggested that apo-IRP1 has an elongated shape.

In this study we analyze small angle X-ray scattering (SAXS) data using *ab initio* calculations to determine the molecular shape of apo-IRP1 in solution, rigid body modeling to assess any differences between its solution conformation and previously determined crystal structures, and the ensemble optimization method (EOM) to investigate its conformational flexibility.

## 2. Results

### 2.1. SAXS, DLS, $R_g$s, and pair distribution functions

SAXS profiles of apo-IRP1 were measured at protein concentrations of 0.54, 1.09, and 2.18 mg/ml at a scattering range ($q$) of 0.065–0.356 $Å^{-1}$ (Fig. 1a). The $R_g$ was determined from solution scattering profiles at a $q \times R_g$ limit of 1.3 using the Guinier approximation (Fig. 1b). At concentrations of 0.54 and 1.09 mg/ml, scattering intensities were linear within the Guinier region, but the 2.18 mg/ml (21.6 μM) samples deviated from linearity, so were not used in further analysis. Subsequent dynamic light scattering (DLS) results showed that apo-IRP1 was monomeric and monodisperse up to the concentration of 1.09 mg/ml (Supplemental Fig. 1). The average $R_g$ was 33.6 ± 0.3 Å (Table 1). This value is much greater than the 28.7 Å calculated from the crystal structure of c-aconitase (PDB ID:

*Abbreviations:* DLS, dynamic light scattering; DTT, dithiothreitol; IRE, iron responsive element; IRP, iron regulatory protein; $R_g$, radius of gyration; SAXS, small-angle X-ray scattering.

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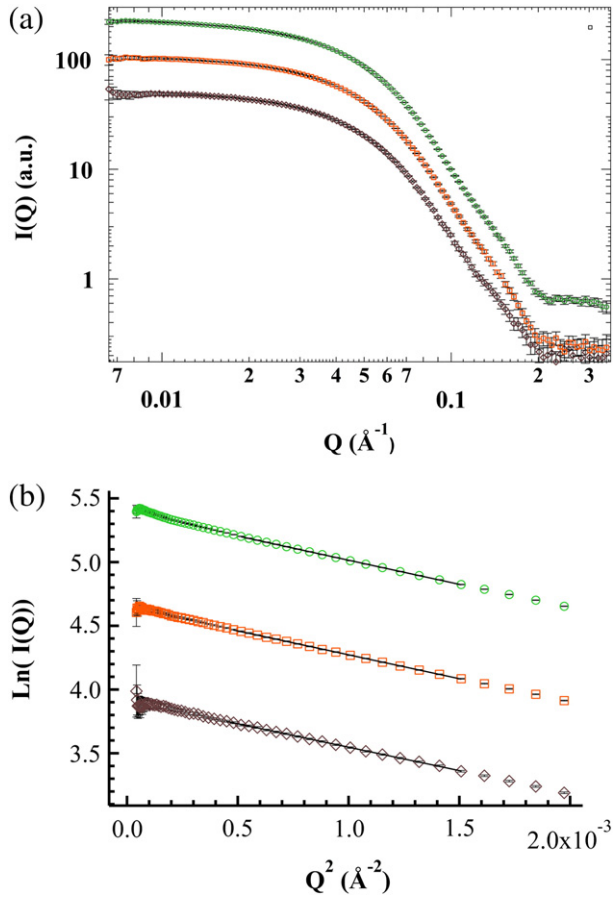


**Fig. 1.** SAXS results. (a) Small-angle X-ray scattering profiles of apo-IRP1 at concentrations of 2.18 mg/mL ○ (green), 1.09 mg/mL □ (orange), and 0.54 mg/mL ◊ (brown). (b) Guinier plots from the solution scattering data of apo-IRP1 at 2.18 mg/mL, 1.09 mg/mL, and 0.54 mg/mL.

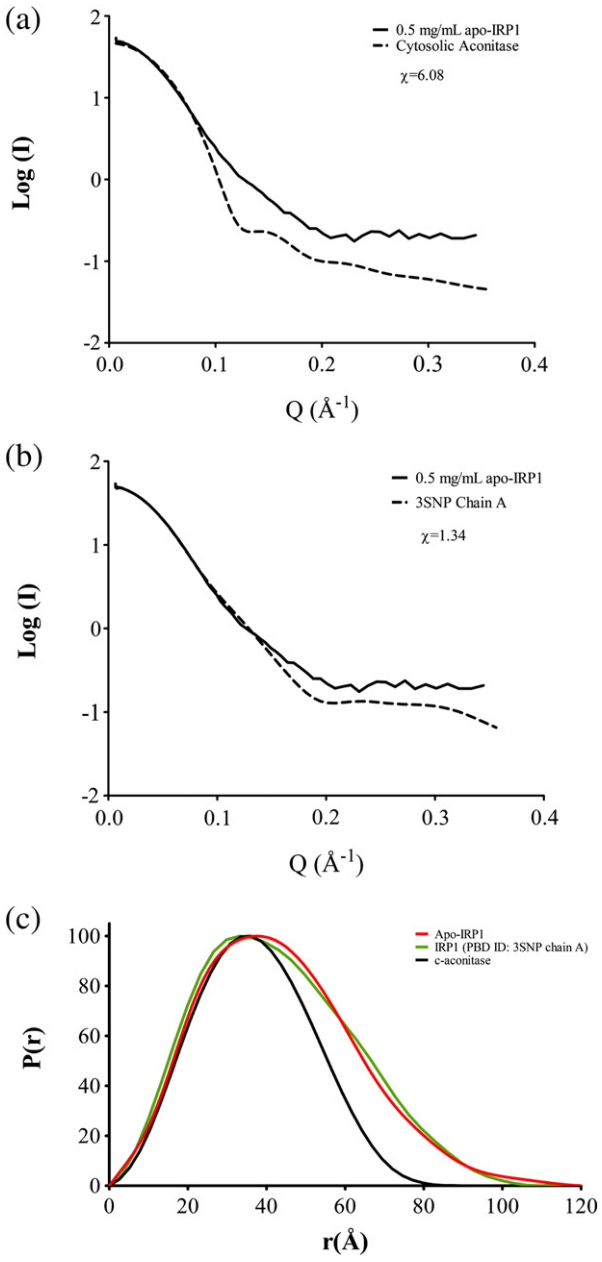


**Fig. 2.** Analysis of SAXS data. The experimental SAXS data for apo-IRP1 compared with theoretical curves of IRP1 in the (a) c-aconitase conformation and (b) RNA-binding conformation using the program CRYSOL. (c) Pair distribution functions calculated from the experimental data for apo-IRP1 (red), the crystal structure of IRP1 when complexed with IRE-RNA (green), and the crystal structure of c-aconitase minus the iron–sulfur cluster (black).

2B3X), but similar to the 33.4 Å calculated from the protein component of the IRP1:IRE-RNA complex (PDB ID: 3SNP). The protein molecular mass ($M_m$) determined directly from the SAXS data by the internal method of Fischer et al. (2010) was 99.2 ± 3.9 kDa, which agrees well with the published value of 100.8 kDa (Selezneva et al., 2006).

When the solution scattering curve of apo-IRP1 is compared to the simulated scattering curve of cytosolic aconitase, differences appear at q values of ~0.08 $Å^{-1}$ and greater (Fig. 2a). The overall goodness of fit ($\chi$) between the apo-IRP1 and cytosolic aconitase curves is 6.08, strongly indicating that the curves arise from molecules with different shapes. In contrast, comparison of the apo-IRP1 scattering curve to the simulated scattering curve of the protein component of the IRP1:IRE complex shows that the scattering curves do not diverge from one another until a q value of approximately 0.13 $Å^{-1}$ (Fig. 2b). The overall $\chi$ value between apo-IRP1 and IRP1:IRE complex is 1.34 suggesting that the two molecules have similar structures.

**Table 1**
Biophysical parameters of IRP1. $R_g$ and $D_{max}$ values for apo-IRP1, IRP1 as bound to IRE-RNA, and c-aconitase. Values for RNA-bound IRP1 and c-aconitase were calculated from crystal structures 3SNP and 2B3X, respectively.

| Protein | $R_G$ (Å) | Dmax (Å) |
|---|---|---|
| c-Aconitase (PDB ID: 2B3X) | 28.7 | 92 |
| IRP1 (PDB ID: 3SNP, chain A) | 33.4 | 108 |
| Apo-IRP1 | 33.6 ± 0.3 | 118 ± 2 |

Pair distribution functions were calculated for apo-IRP1 from the experimental scattering data, and compared with the theoretical curves for IRE-bound IRP1 and cluster-free c-aconitase using the program CRYSOL (Svergun et al., 1995) (Fig. 2c). The pair distribution curve for apo-IRP1 is very similar to that for IRP1 in complex with IRE, and clearly distinct from that for c-aconitase. Apo-IRP1 has the largest $D_{max}$ of 118 Å, whereas $D_{max}$ of the IRE-bound form of IRP1 is next at 108 Å, and c-aconitase has the smallest $D_{max}$ of 92 Å (Table 1).

### 2.2. Ab initio structures of apo-IRP1

Thirty independent *ab initio* models of apo-IRP1 were generated from the SAXS data using the program GASBOR (Svergun et al.,

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