Experimental report

13/09/2019

Proposal: 8-03-968 **Council:** 10/2018

Title: Deciphering the structural organization of the ternary protein complex that switches off photosynthesis

Research area: Biology

This proposal is a new proposal

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Samples: CP12/GAPDH/PRK complex
CP12 protein
GAPDH
PRK

Instrument	Requested days	Allocated days	From	To
D22	1	1	19/07/2019	20/07/2019

Abstract:

By their photosynthetic activity, microalgae are crucial actors in the CO2 pumping for the atmosphere. CO2 assimilation in photosynthetic organisms is performed in the Calvin cycle. A small chloroplastic protein, CP12 regulates two key enzymes of the Calvin cycle, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and phosphoribulokinase (PRK) according to the circadian cycle and its redox state. CP12 contains 4 cysteine residues which form two disulfide bridges when CP12 is oxidized (which corresponds to the night conditions) and is partially disordered. CP12 then binds to GAPDH and PRK to form a ternary complex, thereby inhibiting the Calvin cycle. To decipher the molecular mechanisms of this inhibition, we need to solve the structural arrangement of this complex in solution. SANS with contrast variation is a method of choice for such an issue. SANS experiments on the ternary complex with hydrogenated and perdeuterated subunits of the complex will allow us to reveal the conformation and the respective position of each partner, and to follow in particular the conformational changes undergone by the intrinsically disordered protein CP12 upon complex formation.

Experimental Report : Experiment No. 8-03-968 (performed on 19th July 2019)

Deciphering the structural organization of the ternary protein complex that switches off photosynthesis

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Brief background

We are interested in the mechanisms of regulation of photosynthesis in microalgae via the Calvin cycle, as microalgae are major actor of CO_2 assimilation on Earth. The Chloroplast Protein CP12, an intrinsically disordered protein (IDP) binds and inhibits two key enzymes of the Calvin cycle in oxidized conditions (during the night), and releases them in reduced conditions (during the day), thus allowing the Calvin cycle, and photosynthesis to proceed normally. These two enzymes, PRK and GAPDH form an inactive ternary complex with CP12, once the 4 cysteine residues of CP12 are oxidized and form two disulfide bridges. Because of the disordered nature of CP12 [1,2], X-ray diffraction is excluded to assess the dynamics and molecular mechanisms of formation of this complex. Conversely small angle X-ray and neutron scattering (SAXS and SANS) are particularly well suited to decipher the dynamics and the structural organization of such a ternary complex. To assess the intrinsic dynamics of CP12 within the complex, SANS with contrast variation is a method of choice, that allow to mask the two other proteins from the complex, GAPDH and PRK, and to record the signal from only CP12 in the complex according to the D_2O content of the buffer and the deuteration of GAPDH and PRK.

1. Protocol for the preparation of the samples before data acquisition

A first step of the experiments consisted in setting up the protocol to prepare the protein samples just before data acquisition. A Gel filtration chromatography at 5-10°C was first required, at the EMBL lab, to remove any aggregates that may have formed during sample transportation from the home lab or because of defrosting. A desalting column was also used to change the buffer (H_2O to D_2O) and the buffer redox conditions (presence of DTTox or DTT), knowing that the reducing buffer is stable only for few hours in ambient atmosphere. This protocol allowed us to have a pure protein, and the exact corresponding buffer for SANS measurements. The protein samples were then concentrated to allow high signal-to-noise ratio.

A first series of SANS measurements in oxidized and in reduced conditions showed that the proteins aggregated in the deuterated buffers, but not in the hydrogenated buffers, most probably during the concentration step. We therefore prepared again some fresh sample, in 100% D_2O buffer, by keeping only the top of the elution peak of the gel filtration chromatography, and this time without concentrating the protein sample afterwards. This allowed us to have pure homogeneous protein samples, although less concentrated (1.5mg/mL), but devoid of any protein aggregates.

With this new protocol, the solution of deuterated GAPDH that was left, ended up at too low concentration to allow sufficiently high signal-to-noise measurement. But we now know, for future experiments, which amount of deuterated GAPDH (initial concentration, volume) is required to have a final sample at sufficient protein concentration.

This protocol allowed us to collect nice SANS data on CP12 in four different conditions (oxidized and reduced conditions, 100%H_2O and 100% D_2O buffer). Data were collected at two sample-to-detector distances (2m and 5.6m) and were then merged, in order to cover both the Guinier ranges as well as the higher angles with sufficient statistics.

2. CP12 in reduced conditions ($CP12_{red}$)

The protein CP12 was measured in reduced conditions (presence of DTT), in buffer 100% H_2O and buffer 100% D_2O. The data were compared with the scattering curves obtained from SAXS on SWING beamline at SOLEIL synchrotron, which is equipped with a gel filtration HPLC facility upstream the measurement capillary. The three scattering curves are superimposable (**Figure 1**), meaning first that our samples are of very good quality (no trace of aggregate at all), and that SAS data are reproducible, whatever the scattering particle, X-ray or Neutrons. Only SANS data corresponding to pixels at the edge of the detector ($q>0.24Å^{-1}$) do not perfectly superimpose to the SAXS data, because of geometrical effects that cannot be corrected, according to our local contact Anne Martel.

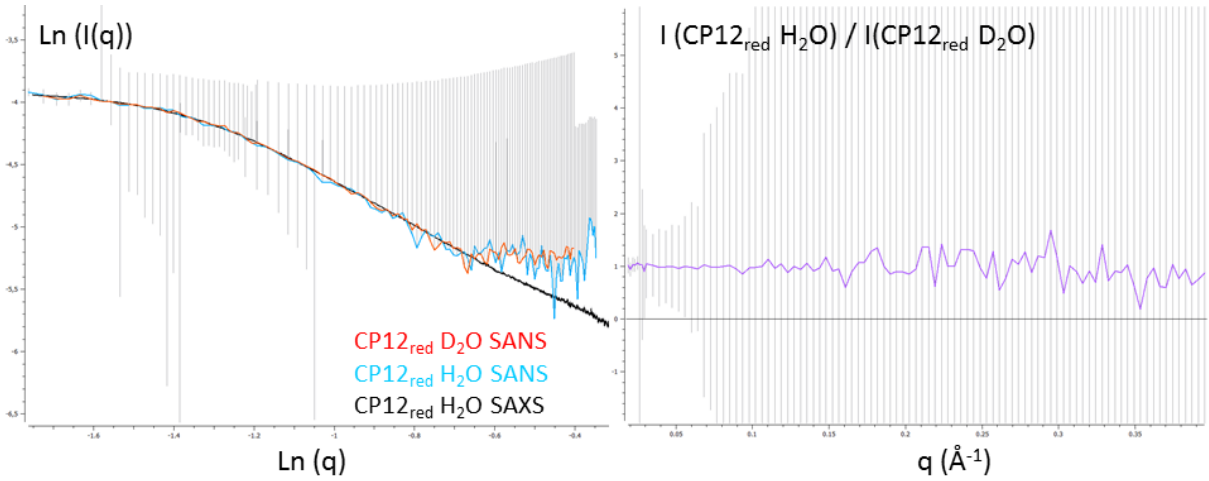

*Fig. 1. Comparison of the SAXS and SANS curves (merged from the data at 5.60m and 2m) of **reduced CP12** in H_2O and in D_2O, showing that they are identical.*

Furthermore, the similar dimensions (Rg, D_{max}, see **Table 1**) obtained in D_2O and in H_2O reveal that the hydration layer, supposed to be visible in H_2O and not in D_2O, is either very thin, or highly dynamic in $CP12_{red}$, which is intrinsically disordered and behaves as a random coil [1]

Table 1. SAXS and SANS parameters measured on $CP12_{red}$ and $CP12_{ox}$ in H_2O and in D_2O buffers

		CP12red		CP12ox	
		Rg (Å)	**D_{max} (Å)**	**Rg (Å)**	**D_{max} (Å)**
H_2O	**H_2O SAXS**	28.3 ± 0.2	110	23.0 ± 0.1	100
	H_2O SANS	28-30	110-120	~ *26*	~ *102*
D_2O	**D_2O SANS**	27 ± 1	100-110	**19.5 ± 1**	70

3. CP12 in oxidized conditions ($CP12_{ox}$)

Measurements were also performed on oxidized CP12, both in 100% H_2O and in 100% D_2O buffers. Comparison of the SANS and SAXS curves confirms the good quality of our samples (no aggregates)

and that the data are reproducible (**Figure 2**). However, $CP12_{ox}$ in D_2O buffer is more compact than in H_2O buffer (**Figure 2** and **Table 1**). This could be ascribed either to a significantly visible hydration layer in H_2O for $CP12_{ox}$, which is partly ordered [2], by contrast with $CP12_{red}$, or to a displacement of the conformational equilibrium between the ordered and the disordered conformations towards more compact conformations [2], because of the more hydrophobic nature of D_2O buffer. Circular dichroism experiments on $CP12_{ox}$ in D_2O buffer will be undertaken in the lab to allow us to discriminate between these two hypotheses.

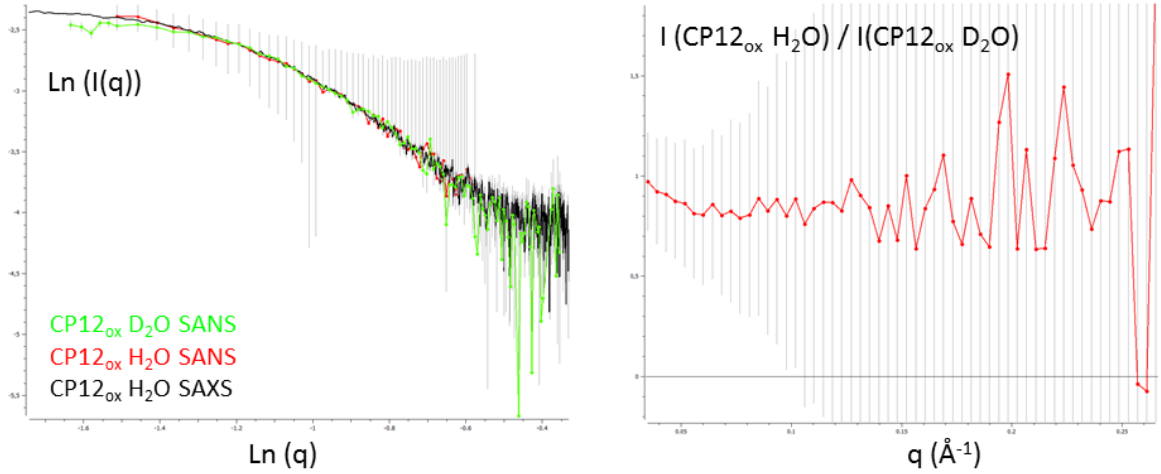

*Fig. 2. Comparison of the SAXS and SANS curves (merged from the data at 5.60m and 2m) of **oxidized CP12** in H_2O and in D_2O, showing that $CP12_{ox}$ in D_2O is . more compact than in H_2O.*

Conclusion

This first series of experiments allowed us to set up the protocol for the preparation on site, after transportation from the home lab, of pure and high quality samples, before the SANS data collection, in both 100% H_2O and in 100% D_2O buffers. We obtained very nice data on CP12 in reduced and oxidized conditions, which showed that the SAS data were reproducible between SAXS and SANS experiments. We could also observe that the conformational behavior of $CP12_{ox}$ in D_2O buffer might be altered possibly because of the higher hydrophobic interactions in this buffer.

While we could produce and purify deuterated recombinant GAPDH at home from *Escherichia coli* cultures, grown in deuterated minimum media, data could not be recorded on this deuterated GAPDH, either isolated or in complex with $CP12_{ox}$ because of insufficient protein concentration after the use of the new sample preparation protocol. Higher amount of deuterated GAPDH are therefore required for future experiments, and we will therefore ask next time for the D-lab for the production of deuterated GAPDH and PRK.

References

[1] Launay H., Barré P., Puppo C., Manneville S., Gontero B., Receveur-Brechot V. (2016), Absence of residual structure in the intrinsically disordered regulatory protein CP12 in its reduced state. *Biochem Biophys Res Commun*, **477(1),** 20-26.

[2] Launay H., Barré P., Puppo C., Zhang Y, Maneville S., Gontero B., Receveur-Brechot V. (2018) Cryptic disorder out of disorder: encounter between conditionally disordered CP12 and glyceraldehyde-3-phosphate dehydrogenase. *J Mol Biol*, **430(8),** 1218-1234.