Analysis of conformational changes of a membrane protein using size exclusion chromatography

This application note describes the use of Superdex™ 200 Increase 5/150 GL column for size exclusion chromatography (SEC), also called gel filtration, in the analysis of conformational changes of the CorA magnesium channel in response to Mg^{2+} binding. SEC allowed for rapid analysis of protein complex formation under non-denaturing conditions. By simply altering buffer conditions, Mg^{2+}-dependent conformational changes could be studied with 20 mutants in a single 24 h experiment. The work was previously published by Palombo *et al*. (1).

Introduction

Studies of membrane proteins have proven challenging due to their hydrophobic nature. Although the structures of some of these molecules have been resolved by X-ray crystallography, conformational changes in response to regulators are difficult to capture using this technique.

SEC elution profiles are commonly used in monitoring of protein interactions and complex formations. Here, we describe how SEC was used in the investigation of conformational changes of the CorA magnesium channel in response to its Mg^{2+} ligand.

CorA is responsible for Mg^{2+} uptake in prokaryotic cells and mitochondria. X-ray structures of CorA from *Thermotoga maritima* (*Tm*CorA) show that this protein is a homopentamer with the ion channel located centrally and the large soluble domain, containing the presumed Mg^{2+} gating sites M1 and M2, located on the cytoplasmic side. The structures indicate that its closed conformation is stabilized by binding of Mg^{2+} to the gating sites M1 and M2. A preserved motif (YGMNFXXMPEL) in the periplasmic loop of CorA, however, has shown to be essential for Mg^{2+} uptake, and it was suggested that the five loops of the pentamer have a role in cation selectivity. To investigate the role of the periplasmic loop in stabilizing the pentamer structure, mutations were incorporated in the loop region and the pentamer formation of the loop mutants in the presence and absence of Mg^{2+} was studied by SEC.

Materials and methods

Wild type (*wt*) *Tm*CorA and 20 loop mutants, each containing one alanine substitution, were expressed as $(histidine)_6$-tagged recombinant proteins in *E. coli*; purified from membrane fraction by capture on a nickel-charged chromatography medium; and stored in 20 mM Tris-HCl, 0.3 M NaCl, 0.03% (w/v) DDM, pH 8 at a concentration of 5 mg/ml.

Before SEC analysis, protein concentrations were adjusted to 0.5 mg/ml in either SEC buffer (20 mM Tris-HCl, 0.3 M NaCl, 0.03% (w/v) DDM, pH 8) or in SEC buffer containing 100 mM $MgCl_2$ and samples were allowed to incubate for about 1 h at 4°C. A sample volume of 25 µl was loaded at a flow rate of 0.3 ml/min onto the Superdex 200 Increase 5/150 GL column connected to an ÄKTA™ system equipped with an autosampler and installed with the UNICORN™ system control software. Eluted protein was monitored at 280 nm. For data analysis, the QtiPlot software was used. Native PAGE analysis was used for confirmation of the results obtained by SEC.

A more detailed description can be found in the publication by Palombo *et al*. (1).

Results and discussion

Figure 1 shows representative examples of SEC elution profiles in the presence and absence of Mg^{2+}. The *wt* protein eluted as pentamer independent of buffer condition.

In the presence of Mg^{2+}, all loop mutants eluted as pentamers. These results confirm previous findings, suggesting that binding of Mg^{2+} to the gating sites M1 and M2 in the cytoplasmic domain induces a closed conformation of the CorA channel.

In the absence of Mg^{2+}, 12 of the loop mutants eluted as pentamers (Fig 2). The remaining eight mutants eluted as monomers or lower oligomers, indicating that these mutations are critical for pentamer formation. Interestingly, seven of the eight mutations are located in the preserved motif (YGMNFXXMPEL) of the periplasmic loop.

Taken together, the data indicate that the pentameric state can be maintained either by the cytoplasmic M1 and M2 gating sites in the presence of Mg^{2+} or through interactions mediated by the periplasmic loops in the absence of Mg^{2+}. The authors propose that these two alternative ways to stabilize the pentameric structure reflect the open and closed states of the CorA magnesium channel.

Automation of the procedure was accomplished by operation of the Superdex 200 Increase 5/150 GL column through an ÄKTA system. The system setup enabled about 70 SEC analyses (each 20 min) to be performed in one 24 h experiment.

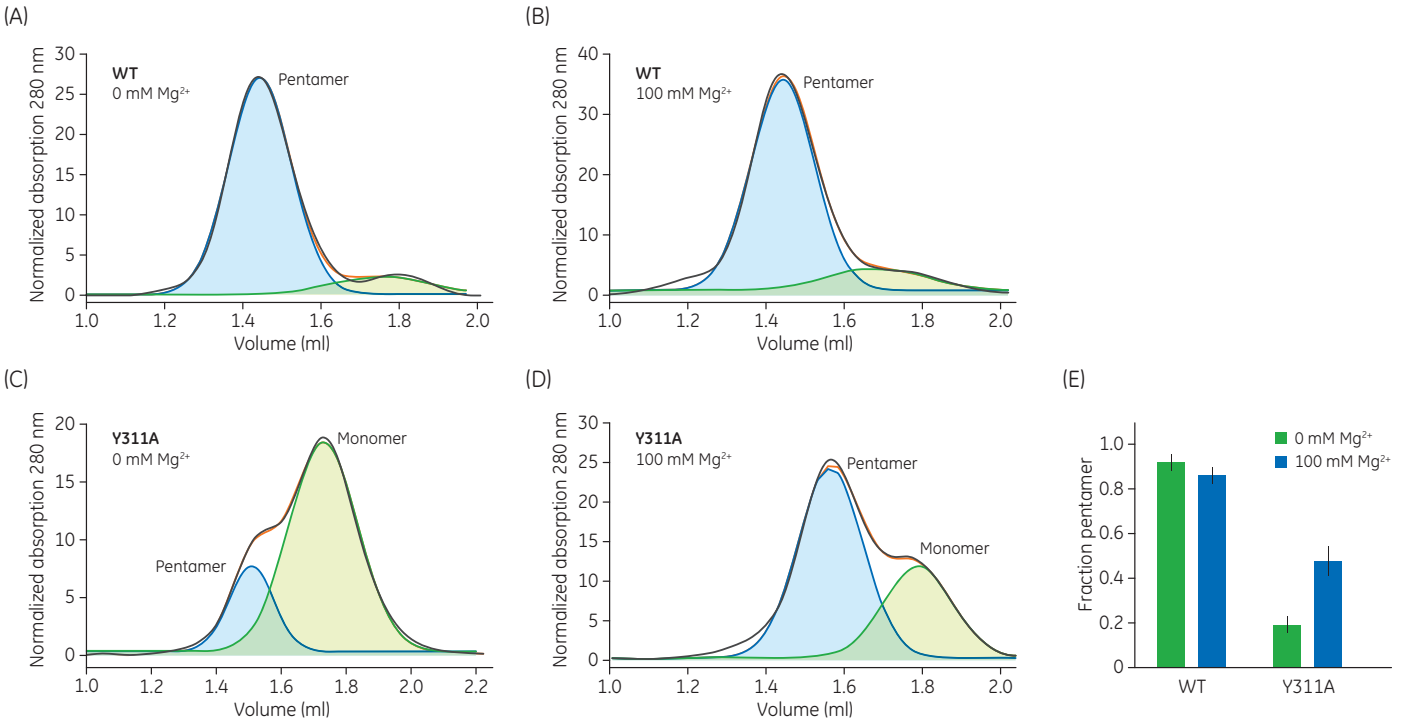

Fig 1. Representative examples of SEC elution profiles in the presence and absence of Mg^{2+}. The *wt* protein predominantly elutes as pentamer in both (A) the absence and (B) the presence of Mg^{2+}. The loop mutant Y311A primarily elutes as monomer in (C) the absence pf Mg^{2+}, whereas in (D) the presence of Mg^{2+}, this same loop mutant primarily elutes as pentamer. (E) Normal distributions were fitted to the elution spectra of monomers and pentamers, respectively, and the pentamer content was calculated as the fraction of the total area (monomer + pentamer).

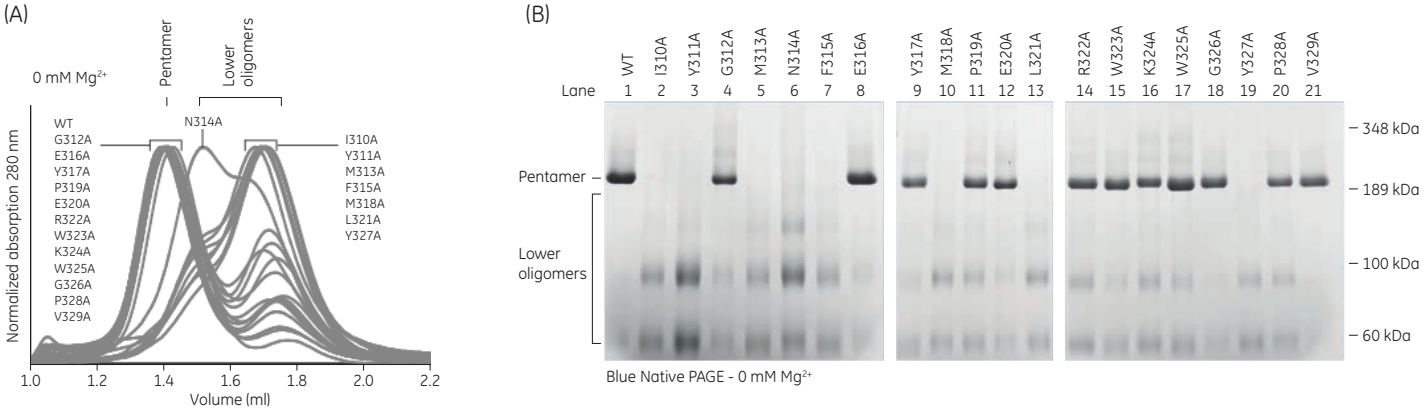

Fig 2. Analysis of pentamer formation in the absence of Mg^{2+} by (A) SEC and (B) native PAGE.

Conclusion

Here, we show how SEC analysis was used in the investigation of the regulation of Mg^{2+} uptake through the CorA channel. SEC analysis using the Superdex 200 Increase 5/150 GL column satisfied a number of important experimental and analytical criteria. Critical for the investigation was that the method enabled separation of mutants that had lost their ability to sustain the native pentameric state from mutants that exhibited a retained pentameric state. The effects of the Mg^{2+} ligand could be investigated by simply altering running buffer conditions. Quantification of experimental data was straightforward. By fitting one or two normal distributions to the elution profile, the pentamer fraction could easily be estimated for each mutant protein. The results indicate that in addition to its role in cation selectivity, the conserved periplasmic loop has a structural role to scaffold the CorA pentamer in an open conformation (*i.e.*, in the absence of Mg^{2+} when the cytoplasmic M1 and M2 gating sites are unoccupied).

SEC analysis using the Superdex 200 Increase 5/150 GL column connected to an ÄKTA system was found to be rapid and robust. The method (20 min) allowed for investigation of the effects of two different buffer conditions on 20 loop mutations in one 24 h experiment.

Acknowledgements

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Reference

1. Palombo, I., Daley, D.O., Rapp, M. The periplasmic loop provides stability to the open state of the CorA magnesium channel. *JBC* **287**, 27547–27555 (2012)

Ordering information

Product	Size	Code number
Superdex 200 Increase 5/150 GL	1	28990945

Related literature	Code number
Data file: Superdex 200 Increase columns	29045269

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