CRF grant ¨Molecular anatomy and physiology of human cystinosin¨

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Topology study of cystinosin

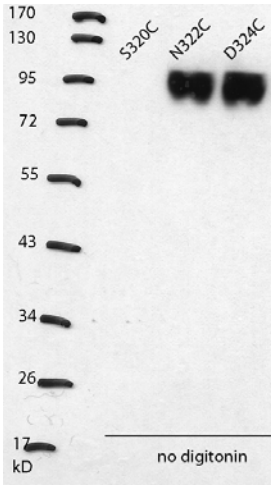
As mentioned in the previous report, scanning cysteine accessibility mutagenesis (SCAM) was established for the study of membrane topology of cystinosin. In our method, EZ-link Biotin-HPDP (Pierce) is used as the biotinylation reagent, and three kinds of cysteine location are distinguishable according to the criteria in Table 1:

Table 1: Criteria used to determine the topological orientation of tested cysteine residues based on their biotinylation behaviour in Xenopus oocytes

Cysteine location	Biotinylation results
Extracellular	Biotinylation without permeabilization
Within lipid bilayer	Little or no biotinylation even after permeablization
Cytosolic	Biotinylation only after permeabilization

Until now, we have tested 75 cystinosin mutants with this method. Our results support the idea that cystinosin contains 7 transmembrane segments, with the C-terminus in the cytosolic side. Most of the 14 borders of these transmembrane segments have been experimentally determined within a window of 3 consecutive residues (e.g., see Figure 1).

Figure 1: SCAM analysis shows that the extracellular border of the 6th transmembrane segment sits between residues S320 and N322.



Some residues predicted to be buried in the lipid bilayer were also investigated. Among them, several turned out to be accessible to the biotinylation reagent, either extracellularly or cytosolically, thus suggesting that these residues are situated along the transport pathway of cystine through the membrane rather than in direct contact with the lipid bilayer. Until now, residues lining the "transport pathway" have been found on 4 transmembrane segments (out of 7), which may constitute the active 'core' of cystinosin. Interestingly, residue D305 is one of them, in agreement with its crucial role in the transport function of cystinosin as demonstrated by a previous electrophysiology study from our group. In addition, using the SCAM-based borders of the corresponding transmembrane segment, we calculated that the relative depth of D305 in the lipid bilayer is 0.7 (with values of 0 and 1 for the lysosomal and cytosolic sides of the membrane, respectively), a value close to the dielectric depth of 0.65 determined by electrophysiological analysis.

We also tested the transport activity of the single-cysteine mutants, using ^{14}C-labelled cystine as the substrate. Some mutants showed significant decrease in cystine transport. We found that mutations in the "transport pathway" often impair transport activity.

To explore the role of the tested residues in substrate binding and translocation, we also used another approach in which Xenopus oocytes expressing the single-cysteine mutant are preincubated with a high concentration of cystine prior to biotinylation analysis. In a primary screen, several mutants showed a significant change in biotinylation level in the presence of cystine. These data need to be confirmed and investigated further, e.g. using TEVC.

In conclusion, our SCAM study of cystinosin has already yielded a comprehensive and accurate map of the topology of cystinosin, including the delineation of an aqueous "transport pathway" across the membrane. Plotting of known pathological mutations onto this experimentally validated map may provide new insight into the molecular pathogenesis of cystinosis. Moreover, additional SCAM experiments performed in the presence of cystine or on voltage-clamped oocytes might unveil which regions of cystinosin undergo conformational changes during the cystine translocation process.