

Conformational dynamics of the full membrane bovine mitochondrial ADP/ATP carrier revealed by hydrogen/deuterium exchange mass spectrometry measurements

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The ADP/ATP carrier catalyzes the transport of ADP and ATP across the mitochondrial inner membrane, thus playing an essential role in the cellular energy metabolism. This protein can be inhibited very specifically by two natural poisons, carboxyatractyloside (CATR) and bongkrekic acid (BA) which stabilize the protein in two distinct conformational states acquired during the transport mechanism. A high resolution X-ray structure was obtained for the CATR-inhibited complex, which has not yet been possible for the BA-inhibited complex. A study of both complexes was undertaken by hydrogen/deuterium exchange mass spectrometry (HDX MS). The local differences in conformation gave new insight in the conformational dynamics of the nucleotide transport mechanism.

A new on-line LC-MS procedure compatible with HDX MS experiments was developed to remove different types of non-ionic detergents from the sample after digestion with pepsin. It is based on selective detergent extraction after capture of the sample on the reversed phase cartridge. The extraction is performed by chlorinated solvents and works well for polyoxyethylene based non-ionic detergents but also for polymers like polyethylene and propylene glycol. Detergent removal can be also carried out on a protein level but a special care must be taken with hydrophobic proteins. In such a case it is preferable to perform detergent removal after proteolysis which digests the protein to peptides and reduces the hydrophobicity. The method can easily be automated.¹

Preparation of the carrier was done in Triton X-100 detergent, which is necessary to maintain the carrier functional and soluble. Using the new procedure it was possible to obtain nearly full sequence coverage. However peptides having sufficient signal and resolution covered bit less, 72 % of the sequence. 37 peptides were used to monitor the H/D exchange kinetics. Several couples of overlapping peptides differing by only one amino acid provided high spatial resolution for some amino acids already studied for their role in ATP transport using classical biochemical methods.

The HDX MS data fit very well with the X-ray structure of CATR-inhibited complex and with the biochemical data previously obtained for both complexes. Furthermore

it was possible to determine the local differences of deuterium incorporation between both inhibited forms of the carrier. Based on the CATR-inhibited structure the HDX data enabled us to propose conformational models of the nucleotide transport through the internal mitochondrial membrane. In this model the carrier would possess a cavity alternatively open to the intermembrane space or to the matrix.

In order to justify our findings and verify the structural differences in natural environment of the protein, the direct deuteration of the mitochondria was undertaken. For this in organello experiment, a new procedure was developed to quickly extract and purify the deuterated carrier in both complexed forms. The optimization of the parameters as well as preliminary data of native H/D will also be presented.

¹Rey et al. (2010) *Anal. Chem.* 82, 5107.