Identifying Pathways out of the Aspartate Binding Pocket of the Phosphoenolpyruvate Carboxylase in C4 Plants

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Phosphoenolpyruvate carboxylase (PEPC) is an important enzyme for the process of carbon-fixation in plants. While in C3 plants carbon is stored in 3-phosphoglyceric acid, in C4 plants carbon is fixated as oxaloacetic acid. Most severe weeds known today are C4 plants. Therefore, inhibiting PEPC of C4 plants is a promising strategy for the development of novel and selective herbicides [1]. The activity of PEPC is negatively allosterically regulated by aspartate [2]. We aim at targeting the aspartate binding site of PEPC by structure- and ligand-based inhibitor design, making use of a recent crystal structure of PEPC from the C4 plant *Flaveria trinervia* [3] and initial hits identified recently [1]. However, visual inspection of the crystal structure revealed a very tight entrance region into the aspartate binding site. We thus investigated possible pathways out of this binding site in order to identify conformational adaptations of PEPC that may facilitate ligand escape and, in turn, to estimate which inhibitor size can still be accommodated for access.

We applied RAMD (Random Acceleration Molecular Dynamics) simulations implemented in NAMD [4] starting from complex models of aspartate or the inhibitor AG1433 [1] bound to PEPC. We performed 100 RAMD simulations of 1 ns length each. We investigated PEPC in the monomeric and dimeric state in order to investigate if and how the oligomeric state influences the escape.

We identified two main pathways out of the binding site, which are differently populated for aspartate and AG1433. The one uninfluenced by a second protomer passes by a positively polarized surface region of PEPC, which suggests favorable access for aspartate that way. Currently, the energetics of ligand access/escape along this pathway is investigated by MD simulations employing umbrella sampling, aiming at computing a potential of mean force.

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