Determination of binding constants for activating ligands of pyruvate decarboxylase from *Kluyveromyces lactis* using SAXS parameters

S. Kutter, R. Golbik, P. Konarev[1], D.I. Svergun[1], S. König

Institute of Biochemistry & Biotechnology, Martin-Luther-University Halle-Wittenberg, Halle (Saale), Germany

[1]European Molecular Biology Laboratory (EMBL c/o DESY), Hamburg Outstation, Germany

Beside the enzymes from *Zymomonas mobilis* and *Neurospora crassa* all other pyruvate decarboxylases investigated so far show allosteric substrate activation behaviour. Artificial substrate surrogates like pyruvamide or methyl acetyl phosphonate are also able to activate the enzyme without being converted. It is known from small-angle X-ray solution scattering experiments as well as from protein crystallographic analyses that at least yeast pyruvate decarboxylases undergo global conformation changes during substrate activation [1,2]. Inactive and activated enzyme states differ in their dimer arrangement within the tetramer. Flexible loop regions close over the active sites during enzyme activation. We have determined the crystal structure of pyruvate decarboxylase from *Kluyveromyces lactis* recently [3]. Interestingly, the crystal structure of this pyruvate decarboxylase species is very similar to that of pyruvamide activated pyruvate decarboxylase species from *Saccharomyces cerevisiae.*

In solution, activation of pyruvate decarboxylase from both species result in significant increases of the R_G values, reflecting the observed oligomer rearrangement.

Here, we describe the dependence of scattering parameters on the concentration of the artificial substrate surrogates pyruvamide and methyl acetyl phosphonate, respectively. Measurements have been performed under optimum conditions for catalytic activity (pH 6.0, 100 mM buffer) at 3 mg/mL. Figure 1 illustrates the dependence of R_G on activator concentration. A clear saturation curve can be recognised. The K_D values determined from these plots are lower than those derived from kinetic studies (for pyruvamide only: 97 mM for pyruvate decarboxylase from *K. lactis* and 44 mM for pyruvate decarboxylase from *S. cerevisiae*). The effect on increasing R_G for the enzyme from *K. lactis* is more pronounced for methyl acetyl phosphonate than for pyruvamide. However, the K_D values are very similar for both activators (10 and 16 mM, respectively). The scattering intensities remain constant over the whole activator concentration range (0.5-100 mM). This means that the presence of activators does not cause oligomer dissociation.

To our knowledge, this is the first case for the direct determination of activator binding constants from measurements of changes of the protein structure.

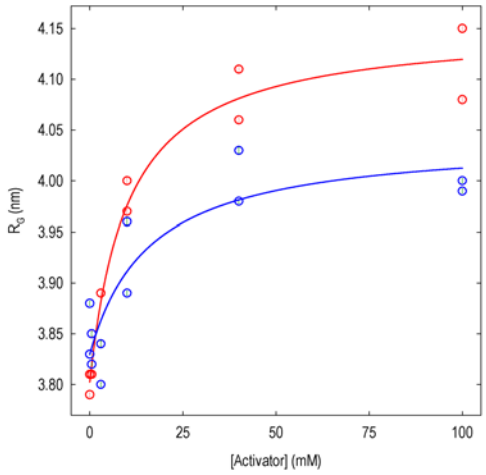

Figure 1: Dependence of the scattering parameter R_G on the concentration of the artificial substrate surrogates pyruvamide (open green circles) and methyl acetyl phosphonate (open red circles), respectively. The lines represent the fits to single rectangular hyperbola with three parameters.

References:

[1] G. Hübner, S. König, A. Schellenberger, and M. H. J. Koch, FEBS Lett. 266, 17 (1990)
[2] G. Lu, D. Dobritzsch, S. Baumann, G. Schneider, and S. König, Eur. J. Biochem. 267, 861 (2000)
[3] S. Kutter, G. Wille, S. Relle, M. S. Weiss, G. Hübner, and S. König, Febs J.: 273, 18, 4199 (2006)