Characterization of Amino Acid tRNA Ligases using the Analytical Ultracentrifuge

fil. lic. Lars Lundvik

GÖTEBORGS UNIVERSITET

Institutionen för kemi och molekylärbiologi

Naturvetenskapliga fakulteten

Akademisk avhandling för filosofie doktorsexamen i kemi med inriktning mot biokemi, som med tillstånd från Naturvetenskapliga fakulteten kommer att offentligt försvaras tisdagen den 29, september, 2015 kl. 13.00 i Hörsal Ragnar Sandberg, Medicinaregatan 7A, Göteborg.

ABSTRACT.

Quaternary structures of amino acid tRNA ligases/synthetases (aaRS) in the native as well as in denatured forms were examined by molecular weight determinations (paper I-IV). Analytical ultracentrifugation, gel electrophoresis, gel chromatography were used in these investigations. The aaRSs were obtained from bacteria and yeast: LysRS and ValRS from S. cerevisiae, AspRS, LysRS and SerRS from E. coli, AsnRS, LysRS, SerRS and ValRS from Bacillus stearothermophilus. The quaternary structures of ValRSs from S. cerevisiae and B. stearothermophilus are both monomeric (the α-type), whereas all the other aaRSs are homodimers (the $\alpha 2$-type). Two of the aaRS-enzymes have also been crystallized. Both of them are LysRS and their structures were examined with X-ray crystallography. LysRS from S. cerevisiae with a resolution of 5 to 6 A in all directions, and from B. stearothermophilus LysRS with a resolution of 8 A.

In Paper V the enzyme HMG-CoA lyase was investigated. The activity of this enzyme is found in Rhodospirillum rubrum cells grown anaerobically in the light with leucine as the carbon source. A 1.2 kb long DNA segment from R. rubrum has been sequenced and includes the first identified gene for a putative 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) lyase, termed hmgL, from a photosynthetic organism.

A parallel project concerned the characterization of a DNA homeobox (HD), where it was shown that the T7 promoter sequence lacked an important guanine for the transcription of this gene.

The analytical ultracentrifugation method as described in this thesis played an important role already when the first protein structures were characterized, and the interest has increased dramatically during the last ten years partly due to automation. I hope that my early work on the application of analytical ultracentrifugation to tRNA ligases/synthetases (aaRSs) also helped to inspire these exciting developments.

Online version

ISBN: 978-91-628-9518-1
Available at http://hdl.handle.net/2077/39312

Print version

ISBN: 978-91-628-9517-4
Printed and bound by Aidla Trading AB, 2015.