Laboratoire de Biochimie Théorique
Institut de **B**iologie **P**hysico-**C**himique
13, rue Pierre et Marie Curie
75005 PARIS

S E M I N A I R E

Mahmudul Hasanad[ad] ,**Sandhya P. Tiwari**[d] , Jesper Z. Haeggström[b], Kenji Mizuguchi[d] and Marjolein Thunnissen[c]

[a]Biochemistry and Structural Biology,Lund University, Sweden
[b]Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden
[c]MAX IV Laboratory,Lund University, Sweden
[ad]Institutefor Protein Research, Osaka University, Japan

« Crystallographic, SAXS and simulationstudies on leukotriene A4 hydrolases revealconformational differencesrelated tocatalytic mechanism «

Vertebrate leukotriene A4 hydrolases (LTA4Hs) are zinc metalloenzymes with an epoxide hydrolase and aminopeptidase activity belonging to the M1 family of aminopeptidases. Bestatin, an amino peptidase inhibitor, can inhibit both activities. The human enzymeproduces LTB4, a powerful mediator of inflammation and is implicated in a wide variety of rheumatoid diseases. The yeast homolog scLTA4H possesses rudimentary epoxide hydrolase activity. Both the structure of the human enzyme and scLTA4H have been solved to investigate the molecular architecture of their active sites with and without inhibitor bestatin.The structure of native scLTA4H shows an open active site. Upon inhibitor binding, a domain shifts occur and the final binding pocket for the aminopeptidase substrate is formed. In the human enzyme the LTA4 binding side is a narrow preformed hydrophobic channel, which protects the labile substrate when bound to the enzyme and no indications of induced fit have been observed. Many members of the M1 family seem to display a certain degree of induced fit, a feature, which however, has never been observed for humLTA4H. To investigate whether the behavior of the human enzyme is due to crystal-system restrictions, small angle X-ray scattering studies have been performed on three members of the family, humLTA4H, scLTA4H and Xenopus (xl) LTA4H. These studies seem to suggest that humLTA4H functions according to a lock-and-key mechanism while the other two members display conformational changes, more fitting with an induced fit mechanism.Predicted SAXS curves from crystal structures of apo and inhibitor bound scLTA4H form shows differences in the high q-angle region. Experimental SAXS data collected from xlLTA4H also shows similar pattern. Using elastic network model-based normal mode analysis, we modeled conformational states that LTA4Hs may adopt and fitted their predicted SAXS curves with the experimental curves. The modeled structures show differences between the homologs, which helps to explain the catalytic differences. This study demonstrates how a hybrid approach can be used when information of an enzyme in its physiological state cannot be obtained by protein crystallography. Furthermore, an accurate explanation of the catalytic mechanism of 3 LTA4H homologs by crystallography, SAXS and simulation will allow us to design drugs that target the enzyme effectively.

Vendredi 9 juin à 14h00
Bibliothèque