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Unraveling the High Catalytic Efficiency of Human Renin with OvineAngiotensinogen and its Application to Clinical Assay

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Background

Renin is fundamental to circulatory homeostasis. Plasma renin has been suggested to be a useful marker for the increased risk of cardiovascular diseases and renal dysfunction. Renin, an enzyme, cleaves its substrateangiotensinogen (ANG) to produce the decapeptide angiotensin I, which is enzymatically converted into the vasoconstrictor peptide angiotensin II.Enzymatic studies showed thathuman renin has a higher affinity (lower K_m)for and higher reaction velocity (higher k_{cat}) with ovine ANG than human ANG[1].Crystal structures of human ANG and human ANG-renin complexreveal substantial conformational changes of ANG (movement of the N-terminus to the active cleft of renin) upon interaction with renin[2,3].To understand the functional difference between human and ovineANGs, we aim to solve three-dimensional structure of ovine ANG through X-ray crystallography.

Materials and methods

Recombinant ovine ANG was expressed in *E. coli* and purified to homogeneity[4]. Sitting drop vapor diffusion method was performed to crystallize ovine ANG using screening kits (Hampton Research).A single crystal was flash-cooled by immersing it into a liquid nitrogen. Diffraction data set was collected on the beamline BL44XU, SPring-8 (Hyogo, Japan). The data was processed by *HKL*-2000. The crystal structure was solved by the molecular replacement method using *MOLREP* using mouse ANG structure (PDB ID: 2WY0) as a template.

Results

Diffraction data were collected at 2.23 Å resolution.The crystal was found to belong the space group $P6_122$ with $a = b = 92.9$ Å, $c = 290.8$ Å, $\gamma = 120^o$. One molecule was found in the asymmetric unit. The model building and structural refinement are in progress.

Conclusion

In clinical studies, ovine ANG has been utilized to measure plasma renin concentration owing to the high catalytic efficiency to human renin [1]. We recently established a novel assay system for measuring renin concentration at picomolar level (normal level of renin in plasma) using recombinant ovine ANG produced in *E. coli* cells[5].The rationale behind the catalytic properties of ovine ANG may be elucidated by a structural comparison between ovine and human ANGs [6].

References

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