

## High resolution structures of the complexes of $\alpha$ -amylase with tripeptides

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### Introduction

The oligopeptides which mimic the function of carbohydrates are called glyco-replica peptides. However, the molecular mechanism of the oligopeptides to mimic carbohydrates is unknown. We have determined the high resolution structures of the complexes of  $\alpha$ -amylase with three tripeptides to investigate the interactions of the tripeptides with  $\alpha$ -amylase.

### Experimental

Three tripeptides, RCA, RSA and KSA, were designed by computer modeling and synthesized by liquid phase synthesis.

The crystals of  $\alpha$ -amylase from *B. licheniformis* were grown by vapor diffusion method. The crystals are 0.5-2.0 mm in length, 0.1-0.15 mm in both width and thickness.

The crystals of the three complexes were prepared by soaking  $\alpha$ -amylase crystals in 10 mM RCA, RSA and KSA, respectively. They were cut to 0.3 mm long and frozen in the presence of 25% glycerol. The X-ray data were collected at station BL6A to 1.75, 1.80 and 1.90 Å resolution respectively. The data were processed using HKL 2000 software, giving  $R_{\text{sym}}$  of 6.7, 7.2 and 7.8 % respectively and data completeness of 100 %.

The structures of the three complexes were solved using difference Fourier method, based on the structure of  $\alpha$ -amylase from *B. licheniformis* [1] (PDB entry 1VJS), and they were refined using program CNS. The peptide fitting was carried out using TURBO-FRODO graphics software.

### Results and discussion

The structures of the complexes of  $\alpha$ -amylase with RCA, RSA and KSA refined at 1.75, 1.80 and 1.90 Å resolution gave R-factors of 19.4, 18.6 and 20.4 %, and free R-factors of 21.4, 20.9 and 22.4 %, respectively.

The tripeptide is bound in the active site pocket of  $\alpha$ -amylase in each complex (Fig. 1). Taken as an example, the temperature factors of the atoms of RCA are in the range of 69-78 Å<sup>2</sup>. The guanidine group of the arginine forms salt bridge and hydrogen bonds to Asp328 of  $\alpha$ -amylase and five water molecules, and some of these water molecules are hydrogen bonded to Glu261 and Asp231 of  $\alpha$ -amylase. Asp328, Asp231 and Glu261 correspond to the reported binding sites in the  $\alpha$ -amylase-acarbose complex structure [2].

### References

- [1] Hwang, K.Y. et al., Mol. Cell, 7, 251-258 (1997).  
[2] Brzozowski, A. M. et al., Biochemistry, 36, 10837-10845 (1997).

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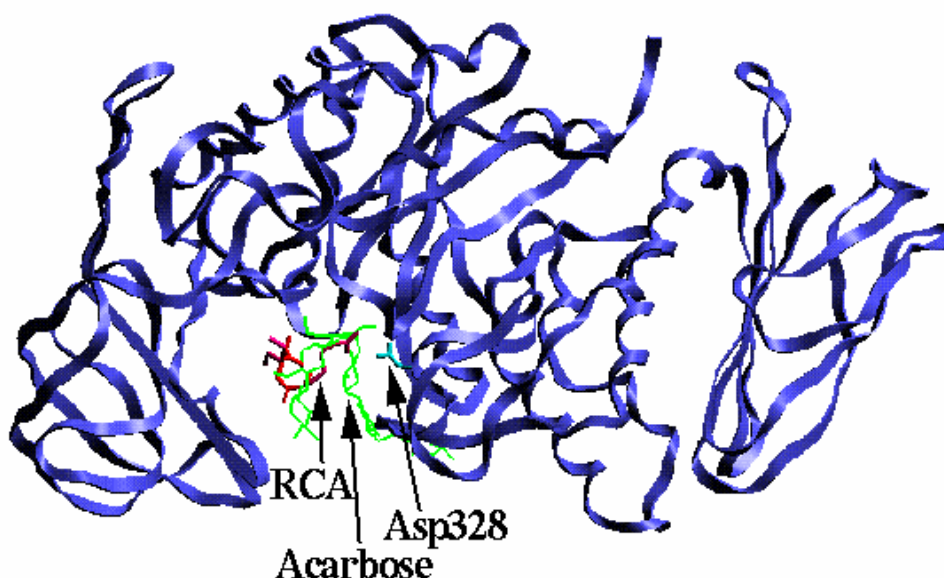


Fig. 1  $\alpha$ -amylase-RCA complex structure.