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Structural change induced by deamidation and isomerization of asparagine as revealed by the crystal structure of *Ustilago sphaerogena* ribonuclease U2B

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Introduction

The deamidation of Asn and isomerization of Asp proceed non-enzymatically *in vitro* and *in vivo*, and result in the formation of isoaspartate (isoAsp) *via* succinimide (Figure 1). The chemical and three-dimensional structural changes caused by these post-translational modification are likely to alter the solubility or bioactivity of the proteins. The alteration *in vivo* could possibly be related to disease, and the alteration *in vitro* could pose a serious threat to the stability of potent proteinaceous biopharmaceuticals. As the three-dimensional structures of the proteins containing isoAsp have been determined in limited number cases, it remains obscure what structural changes take place on protein when isoAsp is formed. We have determined the crystal structure of *Ustilago sphaerogena* ribonuclease (RNase) U2B, whose Asn32 is deamidated and isomerized to isoAsp, at 1.35 Å resolution.

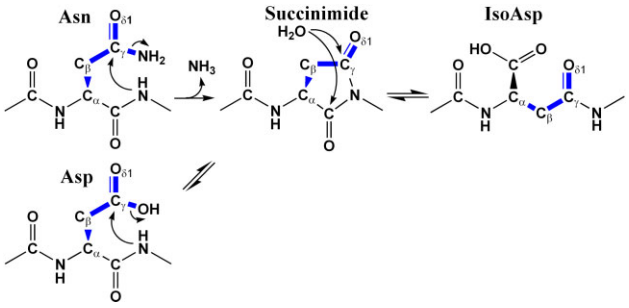

Figure 1. Deamidation and isomerization of Asn. Side-chain bonds of Asn and Asp are colored with blue. Note that succinimide can racemize through keto-enol tautomerism

Experimental Procedure

RNase U2B was crystallized at 293 K by the hanging-drop vapor-diffusion method using the reservoir solution containing NaH_2PO_4 and Na_2HPO_4, pH 6.9. The crystals of a trigonal pyramidal shape appeared within two days. The crystal was cryoprotected by glycerol, and was flash-frozen in a stream of N_2 at 95 K. The data collection was performed at beamline 6A. Diffraction images were processed with the HKL2000 package.

The crystal structure was determined by the molecular replacement method using RNase U2 with normal Asp32 (U2A) complexed with adenosine 3'-monophosphate[1] (PDB code 3AGN) as a search model. Crystallographic refinement was performed using REFMAC. Atomic displacement parameters of the individual atoms were refined anisotropically, and the hydrogen atoms generated at their riding positions were included in the model. Crystallographic R and R_{free} were converged to 16.2% and 17.4% at 1.35 Å resolution (PDB code 3AHS).

Results and Discussion

The asymmetric unit contains three RNase U2B molecules, and isoAsp32 residues of the two molecules are defined in the electron density map (Figure 2*a*), showing L-configuration at the C_α atoms. As compared to 3AGN structure, the single turn of the α-helix from Asp29 to Asn32 in RNase U2A is unfolded in RNase U2B, and the region from Asp29 to Arg35 forms a protruding U-shaped structure (Figure 2*b*). The abnormal chemical structure of the β-peptide bond in the main-chain and the newly-generated α-carboxylate in the side-chain, together with the hydrophobic side-chain of Val31, are completely exposed.

Recently, we have solved the crystal structures of C-terminal polypeptide-binding domain of human heat-shock protein 40, Hdj1, complexed with peptides (PDB codes 3AGY and 3AGZ). The structures reveal that the possible recognition site toward non-native protein substrate could accommodate the hydrophobic polypeptide of 4–5 amino-acid residue length. The partially unfolded RNase U2B, or RNase U2 mutant whose Asp29–Arg35 are replaced with the hydrophobic amino-acid residues, might be utilized as proteinaceous non-native substrate models for Hdj1.

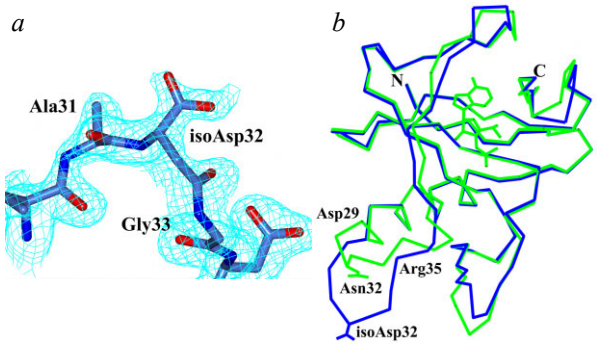

Figure2. (*a*) σ_A-Weighted electron density map at isoAsp32 of RNase U2B. (*b*) C_α-tracings of RNase U2B (blue) and RNase U2A complexed with adenosine 3'-monophosphate (3AGN; green).

References

[1] S. Noguchi, Acta Crystallogr. Sect. D, in press (2010).

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