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STRUCTURE NOTE

A novel mode of dimerization via formation of a glutamate anhydride crosslink in a protein crystal structure

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***Key words:** X-ray; crystal structure; dimerization; glutamate anhydride.*

INTRODUCTION

Acid anhydrides are formed by removal of water, are very active, and can react with water to form the original binary components. Most of the high-energy phosphate-containing compounds are formed by condensation of the phosphate ion with a phosphorylated sugar. The resulting pyrophosphate bond represents a classic anhydride bond. To the best of our knowledge, anhydride bonds formed by condensation of either glutamate or aspartate have not been reported in proteins. A glutamate anhydride can be formed by sharing of OE with another adjacent glutamic acid sidechain, and removal of H_2O, as depicted in Figure 1. Herein, we describe the crystallographic elucidation of a novel glutamate anhydride crosslink between two protein molecules (within a symmetric N^5-glutamine AdoMet-dependent methyltransferase dimer).

The efficient termination of protein synthesis is achieved by post-translational methylation of the Gln residue within the GGQ motif of the so-called ribosome release factors. N^5-glutamine AdoMet-dependent methyltransferase, also known as HemK/PrmC, was the first methyltransferase[1] found to be responsible for the methylation of the highly conserved GGQ motif in class I release factors (RF1 and RF2) in *E. coli*.[2] Class I release factors (RFs) recognize stop signals in mRNAs,[3] RF1 detects UAG and UAA, and RF2 detects UAA and UGA. Both RF1 and RF2 share the tripeptide GGQ motif that is post-translationally modified at Gln to N^5-Me-Gln.[4]

MATERIALS AND METHODS

Protein purification, crystallization, data collection, structure determination, and refinement

Protein expression/purification utilized previously published protocols, which are described in detail in PepcDB (http://pepcdb.pdb.org/). Diffraction quality crystals were obtained at 4°C via sitting drop vapor diffusion of protein previously incubated with AdoMet and Gln (protein concentration ~20 mg/mL) against a reservoir solution containing 30% (w/v) PEG8000, 0.2*M* sodium acetate, 0.1*M* sodium citrate pH 5.6 as precipitant. Crystals appeared in 20–25 days. Crystals were frozen by rapid immersion in liquid nitrogen using the mother liquor supplemented with 15% (v/v) glycerol. Diffraction data were recorded at beamline X12C (National Synchrotron Light Source, Brookhaven National Laboratory) with Brandeis B4 CCD detector and reduced, scaled, and merged using HKL2000.[5] The structure was determined by the molecular replacement

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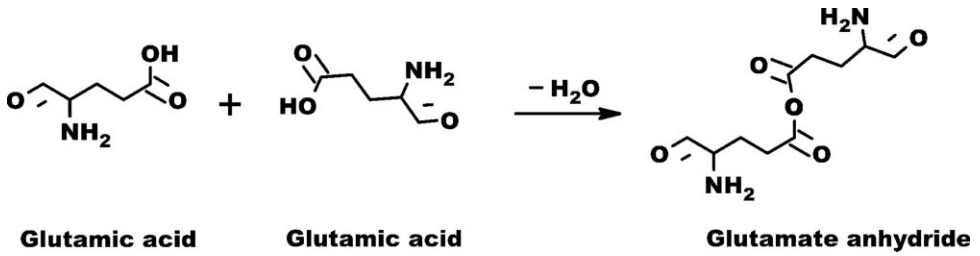

Figure 1
Mechanism of glutamate anhydride formation.

method using one protomer from PDB ID 1NV8 as the search model.[6] The resulting model was refined with CNS[7] at 2.3 Å resolution. The $|F_{obs}| - |F_{calc}|$ difference Fourier map showed two well-defined residual densities, which could be modeled as AdoMet and Gln. Atomic coordinates and structure factors have been submitted to the PDB (PDB ID: 1SG9).

RESULTS AND DISCUSSION

Structural features of N^5-glutamine AdoMet-dependent methyltransferase

Our structure of N^5-glutamine AdoMet-dependent methyltransferase from *Thermotoga maritima* consists of three monomers each with two domains of unequal size [Fig. 2] with two bound ligands *S*-adenosylmethionine or AdoMet and Gln (PDB ID: 1SG9; Space Group: I222). The active site is located within larger of the two domains (N.B.: Given the quality of the electron density corresponding to the bound amino acid, we cannot rule out enzyme catalyzed modification of Gln to N^5-methylglutamine). Similar structures of N^5-glutamine AdoMet-dependent methyltransferase from the same organism were previously described in two distinct ligand-bound crystal forms in space groups C2 (PDB ID: 1NV8; two molecules/asymmetric unit; ligands: AdoMet and N^5-methylglutamine) and $P2_12_12$ (PDB ID: 1NV9; one molecule/asymmetric; ligand: *S*-adenosyl-L-homocysteine) by Schubert.[8] Root-mean-square deviations for all α-carbon pairs are as follows: 1SG9 versus 1NV8 = 1.3 Å, 1SG9 versus 1NV9 = 0.6 Å, and 1NV8 versus 1NV9 = 0.9 Å.

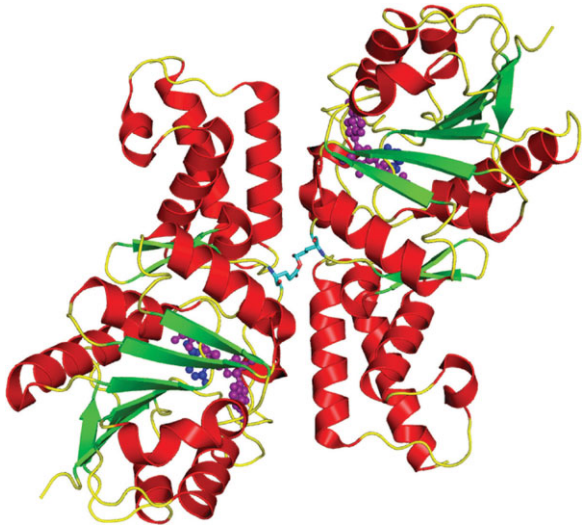

Figure 2
A ribbon representation of the homodimer of N^5-glutamine AdoMet-dependent methyltransferase. Adomet and the substrate Gln are shown in purple and blue, respectively, as a ball and stick representation. Glu97 (Protomers A and C) forming the glutamate anhydride bond are shown in cyan as a ball and stick representation.

A novel mode of dimerization via glutamate anhydride crosslink

In our crystals of *T. maritima*, N^5-glutamine AdoMet-dependent methyltransferase, the asymmetric unit consists of three protomers (A, B, and C). Molecules A and C form a back-to-back dimer with noncrystallographic two-fold symmetry, thereby presenting their two active sites to solvent on opposite faces of the enzyme pair. Molecule B makes a similar back-to-back dimer with its symmetry mate (B′) arranged symmetrically about a crystallographic two fold axis. Glu97 projects into the dimerization interface (Fig. 2). Detailed inspection of the electron density map revealed that the A:C dimer is stabilized, at least in part, by formation of a glutamate anhydride between the two Glu97 sidechains protruding from molecules A and C (Figs. 1 and 3). The torsion angles between the two glutamate residues forming the anhydride crosslink are CG(A)-CD(A)-Oe2(A)-CD(C) = −154° and CG(C)-CD(C)-Oe2(A)-CD(A) = −159°. The electron density of the composite omit map was sufficiently well defined to unambiguously model the glutamate anhydride (Fig. 3). The thermal factors of the atoms in the two glutamate sidechains are comparable to those of other protein atoms

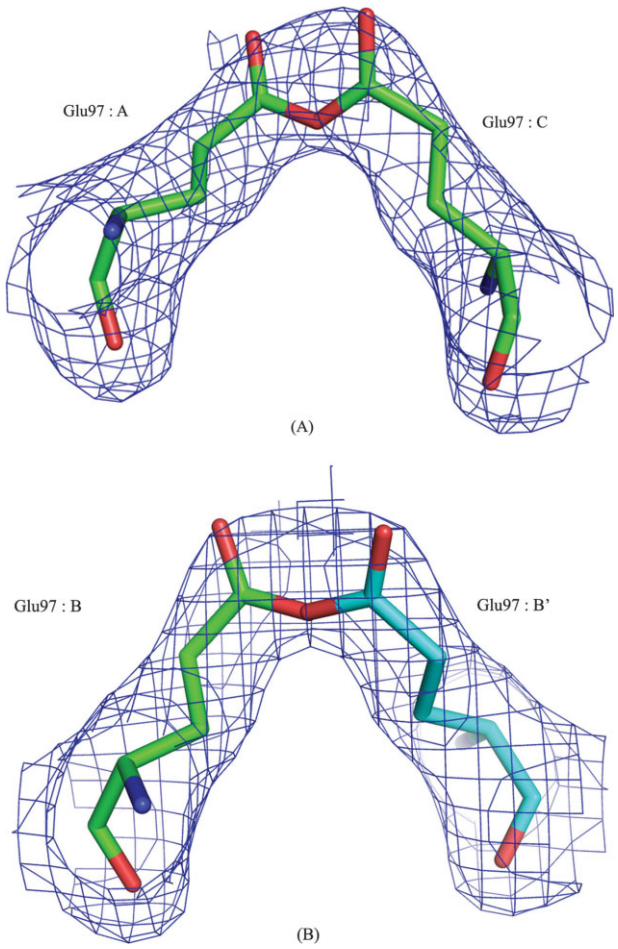

Figure 3

*Composite omit map contoured at 1σ level showing (**A**) Glu97 (Protomers A and C) glutamate anhydride and (**B**) the anhydride formed by Protomer B (green) and its symmetry mate (cyan).*

(Table I). A similar glutamate anhydride link occurs between molecule B and its crystallographic symmetry mate B′. In this case, the inter-protomer torsion angles are CG(B)-CD(B)-Oe2(B)-CD(B′) = −155° and CG(B′)-CD(B′)-Oe2(B)-CD(B) = −172° (N.B.: The electron density for the cross link is also well defined in this case, despite the fact that noise because of Fourier series termination errors can accumulate along crystallographic symmetry axes.). *N*-carboxyl amino acid anhydrides are common in synthetic chemical compounds, but the glutamate anhydride identified herein represents the first time it has been seen in a protein.

The glutamate anhydride crosslink formation

We presume that this anhydride bond formation is favored here because of higher concentration of protein and the enhancement of close proximity via dimerization of molecules. Gel filtration chromatography prior to crystallization demonstrated the presence of both dimer and monomer enzyme populations (data not shown).

Our observation of a glutamate anhydride crosslink in our I222 crystal form of *T. maritima* N^5-glutamine AdoMet-dependent methyltransferase does beg the question as to why the same protein crystallized in two different crystal forms (C2, PDB ID: 1NV8; $P2_12_12$, 1NV9) that did not show the same cross link.[8] Neither of Schubert's crystal forms (C2 or $P2_12_12$) exhibits back-to-back dimerization and the lattice packing interactions bury only minimal solvent accessible surface area. As both 1NV8 and 1NV9 were crystallized at acidic pH using PEG 3K, we cannot readily attribute the behavioral differences among these three systems to differing solution conditions. Instead, we must look to differences in the ligand bound at the active site (AdoMet and Gln/N^5-methylglutamine versus AdoMet or *S*-adenosyl-L-homocysteine).

Table I

Data Collection and Refinement Statistics

Data collection statistics	
Wavelength (Å)	1.1
Resolution (Å)	50–2.3
Space group	I222
Cell dimensions	$a = 81.03$, $b = 188.33$, $c = 207.68$, $\alpha = \beta = \gamma = 90^\circ$
Molecules/asymmetric unit	3
Redundancy	8.0
$I/\sigma(I)$	14.0
R_{merge} [a] (overall/outmost shell)	0.07 (0.62)
Refinement statistics	
Resolution range (Å)	50–2.3
Outermost shell (Å)	2.44–2.3
Number of reflections	63,152
Completeness (%) (overall/outmost shell)	89.1 (90.7)
R_{factor} [b]	0.23
R_{free} [c]	0.26
Number of protein atoms	6494
Number of AdoMet cofactors	3
Number of Gln substrates	3
Number of water molecules	236
Root-mean-square deviation (rmsd) bonds (Å)	0.0075
rmsd Angles (°)	1.43
Ramachandran plot analysis	
Most favored region (additionally allowed) (%)	89.7 (9.2)
Generously allowed region (disallowed) (%)	0.6 (0.6)

Values for the highest resolution shell are given within parentheses.

[a] $R_{merge} = \sum|I_i - \langle I\rangle|/\sum|I_i|$, where I_i is the intensity of the *i*th measurement, and $\langle I\rangle$ is the mean intensity for that reflection.

[b] $R_{factor} = \sum||F_{obs}| - |F_{calc}||/\sum|F_{obs}|$, where $|F_{calc}|$ and $|F_{obs}|$ are the calculated and observed structure factor amplitudes, respectively.

[c] R_{free} = as for R_{factor}, but for 5% of the total reflections chosen at random and omitted from refinement.

The most likely explanation would appear to rest on the active site bound ligands, because Glu97 projects from the face of the protein opposite the active site (Glu97 Cα to AdoMet distance ~11.35 Å). However, it is difficult to offer any specific explanation for this dimerization and we conclude it may the effect of concentration during crystallization.

CONCLUSION

X-ray crystallographically, a novel dimerization of two protein molecules in a structure via glutamate anhydride has been identified for the first time. This crosslink is suggested being an artifact of concentration during crystallization.

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