Communications to the Editor

A Real Knot in Protein

Fusao Takusagawa* and Shigehiro Kamitori

Department of Biochemistry
University of Kansas
Lawrence, Kansas 66045

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It is well-known that circular DNAs exhibit a rich variety of knotted structures.[1] Recent surveys of the X-ray structures deposited in the Brookhaven Protein Data Bank have revealed the presence of pseudolinks and pseudoknots in protein structures caused by formation of disulfide bonds and metal coordination bonds.[2] However, there has been no report so far of knots in native proteins or polypeptides.[2] We now report our finding of a linear knot in the structure of (*S*)-adenosylmethionine synthetase (MAT) recently determined in our laboratory.[3]

During the synthesis of a polypeptide chain in the cell, the newly synthesized peptide typically folds into a globular shape. Perhaps as a result, the polypeptide backbone of any protein has never been observed to form a well-defined knot; that is, if a polypeptide chain were grasped at both ends and pulled straight, a linear chain would always result. A possible exception occurs in carbonic anhydrase where the C-terminus of the chain is somewhat entwined in a large loop.[4] This exception may occur because the very end of polypeptide chain (three residues of the C-terminus) released from the ribosome can fall into a relatively large loop. On the other hand, the N-terminus cannot fold the same way as the C-terminal does since the N-terminus is synthesized in the first place at the ribosome.

To our knowledge, no protein structure determined by X-ray diffraction has one or more knots in either the N-terminal or the middle regions of the polypeptide chain. Thus, the finding of a knot in the polypeptide chain of MAT is dramatic (Chart 1). The knot is made when the B9 β-strand passes through the closed loop composed of the B1 β-strand, the central domain, the B5 β-strand, the H3 α-helix, and the B6 β-strand (Figure 1). If synthesis of the polypeptide chain on the ribosome were immediately followed by the formation of all intrapolypeptide interactions including formation of α-helices and β-sheets, the polypeptide chain of MAT could not fold into the structure found in this study. More specifically, if the B1 β-strand (residues 2−11) formed a stable antiparallel β-sheet with the B6 β-strand (residues 159−172) and consequently formed a small closed loop before the peptide chain of the β-strand B9 (residue 239) was synthesized on a ribosome, then it would be impossible to fold the peptide chain as it is found in the structureof MAT since the ribosome could not pass through the small closed loop. Therefore, in order for the polypeptide chain to fold into the structure observed in MAT, the interactions between B1 and B6 must occur after synthesis of the polypeptide chain of the B9 region, as shown in Figure 2. The structure of MAT suggests that some of the interpeptide interactions, even the formation of an antiparallel β-sheet, do not always occur as the polypeptide chain is synthesized.

In the cell, protein folding occurs not as an isolated event but as one of a set of linked and overlapping processes including translation, translocation, proteolytic processing, posttranslational modification, assembly, and association with ligand or cofactor. The temporal, spatial, and causal relationships be-

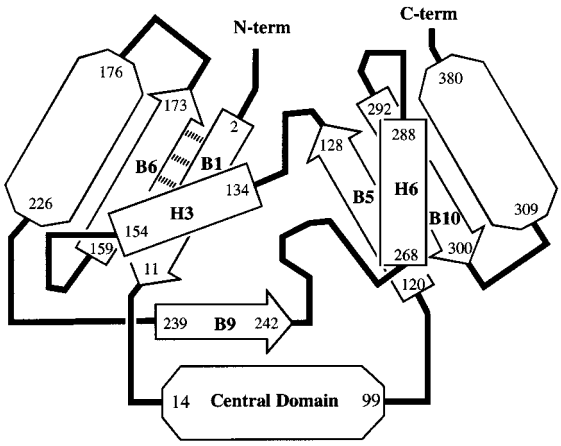

Figure 1. Schematic drawing of the unusual knot structure of the polypeptide chain of MAT. The knot is formed by passage of the B9 β-strand leading to the C-terminus through a loop formed by the sequence B1 → [central domain] → B5 → H3 → B6. The rectangles, arrows, and elongated octagons represent α-helices, β-strands, and portion of domains, respectively. The numbers at both ends are the start and end of the amino acid residue numbers. Hydrogen bonds between B1 and B6 β-strands are shown by dotted lines.

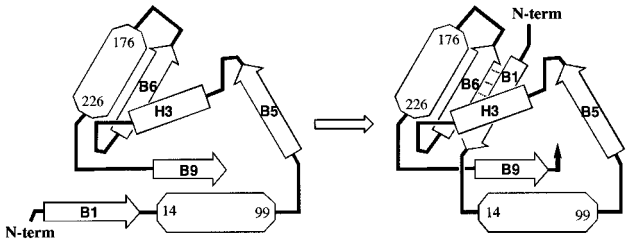

Figure 2. Hypothesis for biosynthetic knot formation. The N-terminal region (residues 1−11) locates near the central domain until after the synthesis of the B9 β-strand (residue 242). After synthesis of the B9 β-strand region, the N-terminal region moves into the closed loop, and the B1 β-strand (residues 2−11) forms antiparallel β-sheet hydrogen bonds with the B6 β-strand shown by dotted lines.

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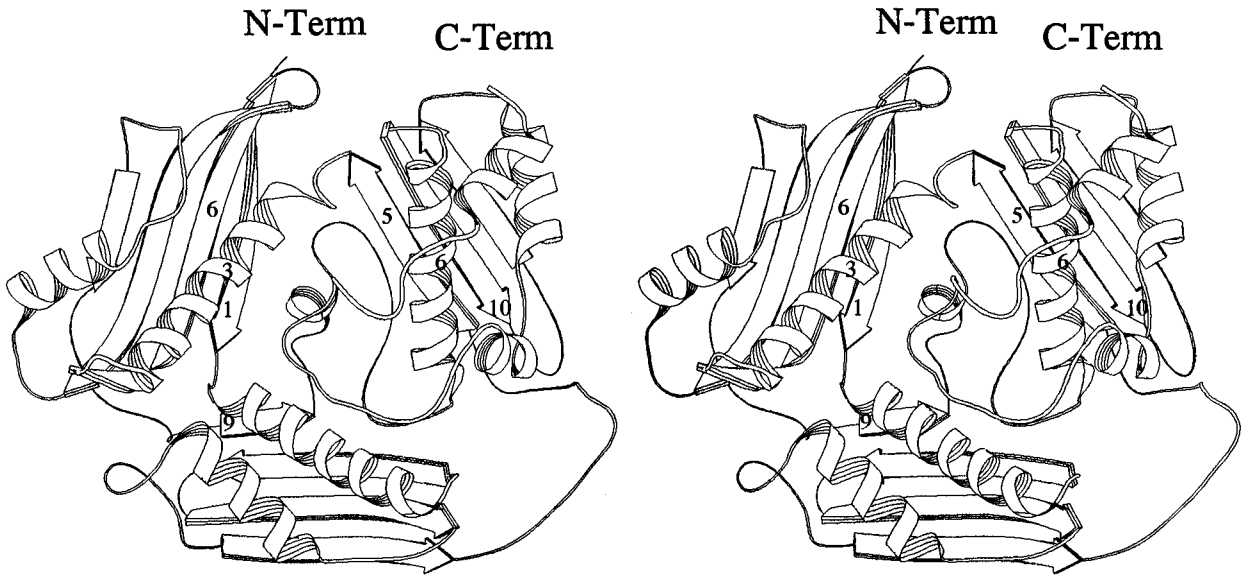
Chart 1. Ribbon Presentation of the MAT Subunit[a]



[a] Numbers of the α-helices and β-strands referred to in the text are marked on the corresponding ribbons.

tween these processes are complex and, in some cases, difficult to resolve. Various studies on protein folding in the cell haveindicated that the vectorial nature of protein synthesis does not determine the outcome of protein folding.[5] In this view, a knot formed at the N-terminus found in the MAT structure may not be unusual. Although the peptide chain does not form knots, Liang and Mislow have recently found the knots formed through disulfide bonds, hydrogen bonds, and coordination bonds in structures in the Protein Data Bank file.[2]

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