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Research paper

Protein unfolding is essential for cleavage within the α-helix of a model protein substrate by the serine protease, thrombin

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ABSTRACT

Proteolysis has a critical role in transmitting information within a biological system and therefore an important element of biology is to determine the subset of proteins amenable to proteolysis. Until recently, it has been thought that proteases cleave native protein substrates only within solvent exposed loops, but recent evidence indicates that cleavage sites located within α-helices can also be cleaved by proteases, despite the conformation of this secondary structure being generally incompatible with binding into an active site of a protease. In this study, we address the mechanism by which a serine endopeptidase, thrombin, recognizes and cleaves a target sequence located within an α-helix. Thrombin was able to cleave a model substrate, protein G, within its α-helix when a suitable cleavage sequence for the enzyme was introduced into this region. However, structural data for the complex revealed that thrombin was not perturbing the structure of the α-helix, thus it was not destabilizing the helix in order to allow it to fit within its active site. This indicated that thrombin was only cleaving within the α-helix when it was in an unfolded state. In support of this, the introduction of destabilizing mutations within the protein increased the efficiency of cleavage by the enzyme. Our data suggest that a folded α-helix cannot be proteolytically cleaved by thrombin, but the species targeted are the unfolded conformations of the native state ensemble.

1. Introduction

Proteolysis has an important role in the transmission of information within a biological system, and is critical in regulating apoptosis and the blood coagulation system, for example [1,2]. An important biological question is to understand which proteins in the cell are capable of being cleaved by proteases. Several key factors regulate the intracellular substrate repertoire of proteases, including cellular co-localisation, exosite affinity, subsite cooperativity and the structural presentation of substrates.

Proteases have long been thought to cleave substrates in flexible solvent-exposed loops arranged in an extended beta-strand conformation [3], or in a higher energy form (an unfolded or partially-unfolded species). However, there are limited structural descriptions of protease-substrate complexes that include the cleavage site residues. A significant point of contention is whether proteases are able to cleave within areas of substrates that are not extended loop regions. Recent bioinformatic and proteomic evidence [4], identifying the secondary structures within substrates of proteins in an *Escherichia coli* lysate preferred for cleavage by caspase-3 and endoproteinase GluC, suggests that a significant number of cleavage sites are present within primary sequences corresponding to α-helical elements in the protein's structure. A further study showed that apoptosis-induced caspase cleavage of substrates in mammalian cells occurs most commonly in loop regions, but a significant proportion of cleavage sites were also observed in α-helices [5]. While it has long been thought that α-helices/structured regions of proteins are resistant to cleavage, these studies have raised the distinct possibility that α-helices and potentially other structural motifs can be recognized and/or cleaved by some proteases.

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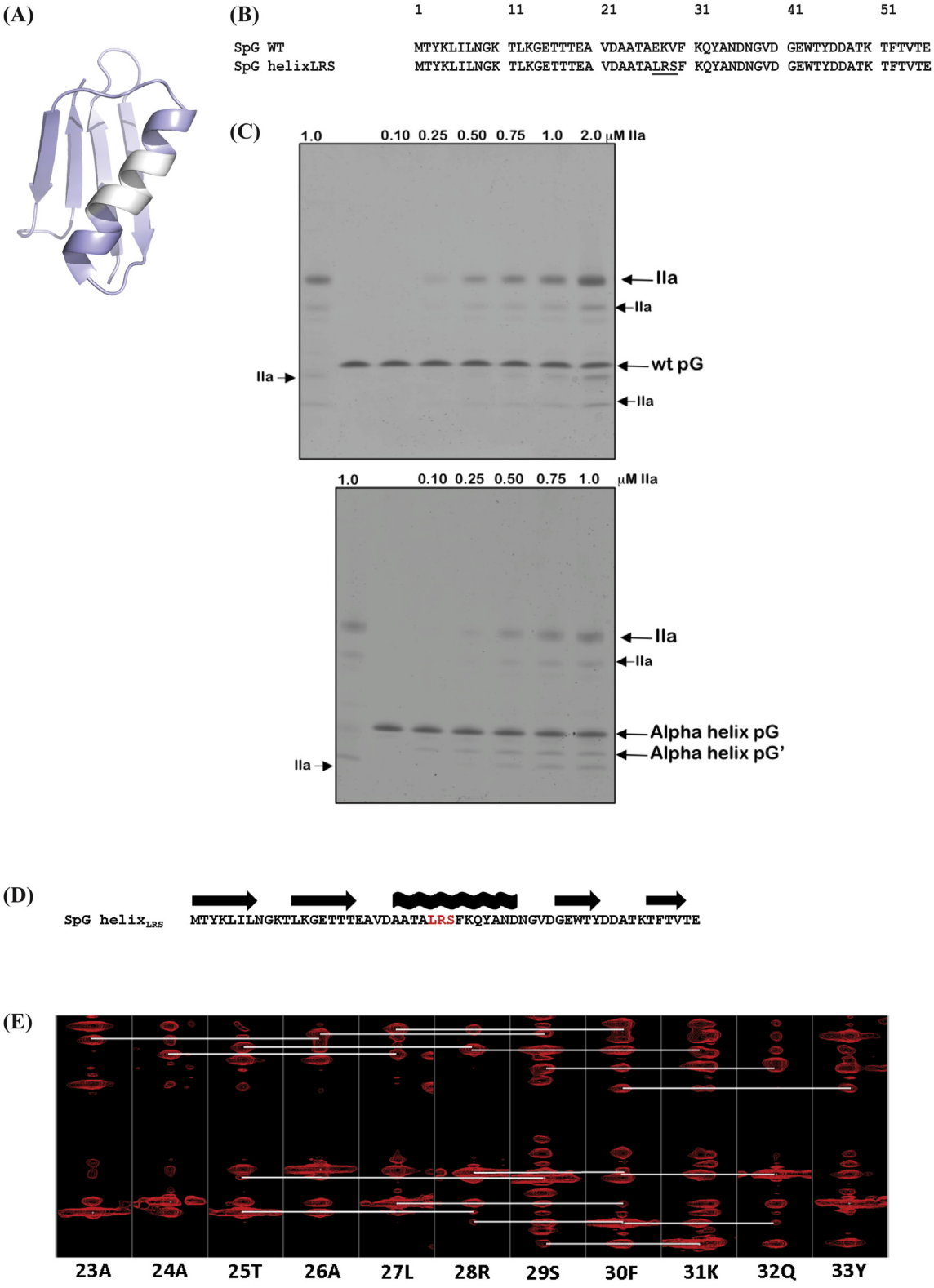

Fig. 1. Effect of inserting P_2–P_1′ thrombin cleavage sequence into the α-helix of SpG. A) The structure of SpG (PDB ID:1GB1). The region highlighted in white is the site at which the P_2–P_1′ LRS sequence was inserted to derive the SpG helix$_{LRS}$. B) The sequence of SpG WT compared to the SpG helix$_{LRS}$, with the substituted residues underlined. C) Thrombin cleavage of SpG WT and SpG helix$_{LRS}$ assessed by SDS-PAGE. Note that in the gel for the SpG helix$_{LRS}$ mutant, the higher molecular weight band that appears is from thrombin. D) A schematic showing the secondary structure prediction of SpG helix$_{LRS}$ determined using TALOS. E) ^{15}N-edited NOESY-HSQC of the H^N and H^α region of SpG helix$_{LRS}$ highlighting the characteristic α-helical NOE patterns.