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Dynamic activation of apoptosis: conformational ensembles of cIAP1 are linked to a spring-loaded mechanism

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Abstract

cIAP1 undergoes a dramatic conformational change during activation that is now shown to be due to the dynamic and metastable nature of the closed form of the enzyme. The discovery of such a striking mechanism for functional control was enabled by state-of-the-art enzymological and biophysical methods.

Over the past two decades, advancements in experimental and computational biophysical methods have provided direct evidence that mechanisms associated with enzyme function are considerably more complicated than the binary switches often depicted in textbooks. Instead, function is modulated by a mixture of conformational states whose relative populations are subject to a complex set of kinetic and thermodynamic controls. Teasing apart these controls is critical both for understanding of biology and for rational drug design. In this issue, Fairbrother and co-workers¹ demonstrate that activation of cellular inhibitor of apoptosis protein-1 (cIAP1) is a particularly striking example of this paradigm. Using a sophisticated combination of enzymology and both static and time-resolved biophysical techniques, they find that the closed form of cIAP1 consists of heterogeneous conformers delineated by domain motions on multiple timescales. These motions are proposed to contribute to a metastable closed state, which enables a 'spring-loaded' conformational change that is required for activation and is quite remarkable given both its magnitude and rate.

Apoptosis is a critical cellular response to both extrinsic and intrinsic signals. It results in the activation of cysteine-dependent aspartyl-specific proteases (caspases), which in turn cause proteolysis and eventually cell death. The activity of caspases is inhibited through various mechanisms by the inhibitor of apoptosis (IAP) family of enzymes, which includes cIAP1. IAPs are commonly overexpressed in cancer cells, where they may contribute to resistance to cancer therapies, thus making them an important pharmaceutical target^{2,3}.

cIAP1 is also a ubiquitin ligase (E3), and, like other E3s, it contains various domains associated with functional regulation (Fig. 1a). In the absence of an apoptotic signal, cIAP1's caspase activation and recruitment domain (CARD) inhibits caspases². When

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proapoptotic antagonists, such as second mitochondrial activator of caspases (SMAC), bind to one of the baculovirus IAP repeat (BIR) domains of cIAP1, two downstream events happen. First, caspases are released to perform proteolysis; second, autoubiquitination of cIAP1 occurs, causing it to be degraded by the proteasome^{4,5}.

The network of domain reorganizations necessary for cIAP1 activation provides an initial hint that conformational dynamics and allostery are critical to enzyme function, as has been reported for other E3s^{6,7}. Further indication of the necessity for conformational dynamics is found in the crystal structure of closed (apo) cIAP1, previously determined by Fairbrother and co-workers⁸. In this structure, the RING interface, which is a dimerization interface for other E3s, and the antagonist-binding site in BIR3 are obscured. The first occlusion would interfere with both E2 recruitment and dimerization upon activation⁶ and the latter with SMAC binding. Because these are critical aspects of the reaction cycle, a triggering mechanism must exist. Using biochemical and enzymological assays, the current work demonstrates that closed cIAP1 is more dynamic than initially posited from the crystallographic structure. Specifically, the affinity of cIAP1 for ubiquitin-charged E2 is shown to be independent of the presence of CARD or the enzyme's oligomeric state. Likewise, SMAC mimetics bind both the closed form and a constitutively open mutant of cIAP1 with similar rates.

To achieve a quantitative and structural understanding of these putative domain motions, the authors use a combination of static and time-resolved NMR spectroscopy and small-angle X-ray scattering (SAXS) techniques. NMR lineshape analysis indicates that microsecond timescale dynamics are present along the CARD-RING interface. NMR spin-relaxation experiments reveal motions of the ubiquitin activating (UBA) domain on the millisecond timescale. Using SAXS, the authors reveal the relationship of these motions to global conformation, with constitutively open and dimerization-deficient mutants allowing models of the closed, open and dimeric enzymatic states to be determined. Additionally, the CARD, in contrast to the other domains, is found to be highly mobile in the closed state, as determined by biochemical and NMR experiments. Finally, the incorporation of time-resolved SAXS measurements and stopped-flow injection enables separation and measurement of the various transition rates, thus demonstrating that opening of cIAP1 is the rate-limiting step for enzyme activation (Fig. 1b).

The conformational change in cIAP1 before dimerization is rivaled in magnitude and rate by only a handful of other biological examples^{9,10}. cIAP1 opening exposes over 8,000 Å² of buried surface area, spans some 20 Å and occurs at a rate of 10 s⁻¹. The result of a transition of this nature is ideal from a functional standpoint: rapid downstream signaling is enabled while rigid control is simultaneously maintained over a cellular process as important as apoptosis. cIAP1 has effectively been tuned to function as a binary switch through a complex dynamic release mechanism to drive enzymatic function. This finding hints at the intriguing possibility of regulating this process via a small-molecule interaction.

The discovery by Fairbrother and co-workers¹ of a mechanism with such an intricate, multistage level of control over biological states as that of cIAP1 activation was enabled by a powerful and diverse arsenal of biophysical methods. These results reinforce three important

observations relating to the biophysical study of macromolecules. First, the most functionally interesting aspects of biology often involve heterogeneous systems and complex motions, which can be masked or invisible within the confines of a crystal lattice. Although crystallographic models have undoubtedly provided a breadth of information about biological systems, a sophisticated understanding of enzymology requires dynamic information. Second, a complete understanding of biological motions requires measurements that are both large in scope and high in resolution on both temporal and spatial scales. In many cases, this necessitates the use of multiple complementary techniques for studying dynamics, as was exquisitely demonstrated for cIAP1. For example, NMR spin-relaxation experiments enable quantitative determination of conformational dynamics on timescales spanning over 12 orders of magnitude with atomic resolution, whereas time-resolved SAXS is more efficient than NMR at determining enzyme global conformation in the presence of multiple states. Finally, the work supports the imperative to draw on the complete array of techniques available as well as to both improve existing methods and develop new ones. The desire for a mechanistic understanding of biology out-strips the limits of current biophysical tools. To enable the discovery of other new conformational mechanisms and inform pharmaceutical drug development, researchers must continue to push the boundaries of these techniques.

The description of the dynamic activation pathway for cIAP1 is an elegant and fore-shadowing application of these concepts. The regulation of downstream signaling through dynamics is an exciting and new perspective on apoptosis, and the possibility to develop modulators acting through dynamics rather than competition or inhibition of enzymatic function may be transformative in many aspects of drug discovery.

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Figure 1.

Structure of cIAP1. **(a)** cIAP1 is composed of multiple domains associated with its function as a caspase inhibitor: baculovirus inhibitor of apoptosis protein repeat (BIR3) domain, ubiquitin activating (UBA) domain, caspase activation and recruitment domain (CARD) and really interesting new gene (RING) domain. **(b)** Activation of cIAP1 is associated with a rate-limiting spring-loaded conformational change that converts the enzyme from the closed (blue) to the open (pink) state. After the conformational change, dimerization of cIAP1 occurs (purple).