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How often does the myristoylated N-terminal latch of c-Abl come off?

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Abstract The myristoylated N-terminal latching to the C-terminal lobe of c-Abl was recently demonstrated to be an important regulatory element for the kinase, playing a role similar to that of the tyrosine-phosphorylated C-terminal tail of c-Src. A potential mechanism for activating c-Abl is the dissociation of the myristoylated N-terminal latch. How often does this latch spontaneously come off? A recent theoretical model along with the experimental results of Superti-Furga, Kuriyan, and co-workers suggests that the equilibrium fraction of c-Abl in which the myristoylated N-terminal is unlatched is ~0.5%.

Key words: c-Abl kinase; Kinase activation; Intramolecular binding; Flexible linker; Effective concentration

1. Introduction

Recent experiments have uncovered an important regulatory element of the c-Abl kinase, in the form of the myristoylated N-terminal latching to the kinase C-terminal lobe [1–3]. The myristoylated N-terminal latch plays a role similar to that of the tyrosine-phosphorylated C-terminal tail of c-Src [4]. One potential mechanism for activating c-Abl is the dissociation of the myristoylated N-terminal latch. How often does this latch spontaneously come off? A recent theoretical model [5,6] along with the experimental results of Superti-Furga, Kuriyan, and co-workers [2,3] provides an answer.

2. Theory

The myristoylated N-terminal must maintain a delicate balance between the latched and unlatched states. If it never becomes unlatched, then c-Abl potentially loses tyrosine kinase activity. On the other hand, if it becomes unlatched too often, then the tyrosine kinase activity may approach that of the fusion protein Bcr-Abl and potentially leads to chronic myelogenous leukemia. The balance between the latched and unlatched states is measured by the association constant K_a^i for intramolecular binding (Fig. 1A).

Previously I developed a theory relating K_a^i to the association constant K_a for the same binding process occurring in a bimolecular fashion [5,6]. Bimolecular reaction results from breaking the peptide linker (residues G2 to N80) between the myristoyl group and the SH3 domain of the tyrosine kinase (Fig. 1B). Hantschel et al. [2] measured K_a using a myristoylated c-Abl N-terminal peptide and found $1/K_a = 2.3$ μM. The relation between K_a^i and K_a is given by

$$K_a^i = K_a p(d) \quad (1)$$

where $p(d)$ is the probability density for the peptide linker (assumed to be flexible) to have an end-to-end distance d, which in turn is the distance between the myristoyl group and residue D81 in the structure of c-Abl in the latched state. The ratio K_a^i/K_a, which equals $p(d)$ according to Eq. 1, is called the effective concentration. A flexible peptide linker consisting of L residues can be modeled as a worm-like chain, such that [7]

$$p(r) = (3/4\pi l_p l_c)^{3/2} \exp(-3r^2/4l_p l_c)(1 - 5l_p/4l_c + 2r^2/l_c^2 - 33r^4/80l_p l_c^3 - 79l_p^2/160l_c^2 - 329r^2 l_p/120l_c^3 + 6799r^4/1600l_c^4 - 344lr^6/2800l_p l_c^5 + 1089r^8/12800l_p^2 l_c^6) \quad (2)$$

where $b = 3.8$ Å is the nearest C_α–C_α distance, and $l_c = bL$ and $l_p = 3.04$ Å are the contour length and persistence length, respectively, of the peptide linker.

3. Results and discussion

From the c-Abl structure [3] in which the myristoylated N-terminal is latched (Protein Data Bank entry 1opl, see Fig. 1C), the distance between the myristoyl group and residue D81 is found to be $d = 56.8$ Å. The assumption of linker flexibility is consistent with the fact that none of the residues in the linker was visible in the X-ray structure of c-Abl. For a peptide linker with $L = 79$ residues, Eq. 2 predicts an effective concentration $p(d) = 0.5$ mM. With $1/K_a = 2.3$ μM, Eq. 1 predicts $K_a^i = 217$. The equilibrium fraction of c-Abl in which the myristoylated N-terminal is unlatched is thus 0.46%. This low unlatched fraction explains the much lower kinase activity of wild-type c-Abl relative to the G2A mutant as observed by Hantschel et al. [2], which is not myristoylated.

The length of the N-terminal linker plays a critical role. Fig. 1D shows the dependences of the effective concentration and the unlatched fraction on the linker length. When 45 residues are deleted from the linker (corresponding to the ΔL1-5 mutant of Hantschel et al. [2]), the effective concentration reduced to 36 μM and the unlatched fraction increased to 6%, still relatively low. However, when 54 residues are deleted (corresponding to the ΔL1-6 mutant of Hantschel et al.), the effective concentration reduced to just 1 μM and the unlatched fraction became 64%. These predictions are consistent with the kinase activities of the ΔL1-5 and ΔL1-6 mutants

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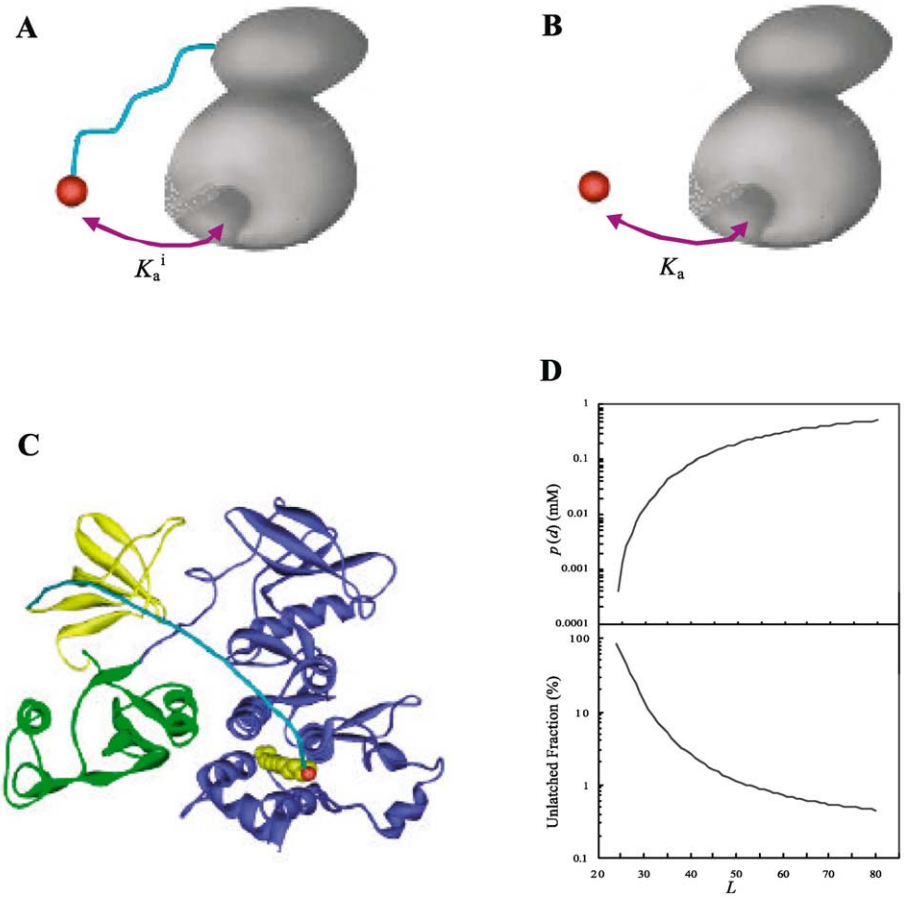

Fig. 1. Binding of a latch to tyrosine kinase. A: The latch is tethered to the kinase by a peptide linker. The association constant is K_a^i. B: The latch is untethered. The association constant is K_a. C: The structure of c-Abl in which the myristoylated N-terminal is latched to the kinase. The peptide linker (in cyan) connected to the myristoyl group (with atoms shown as red and yellow spheres) was not observed by X-ray diffraction [3]. D: The dependence of the effective concentration $p(d)$ and the unlatched fraction on the linker length. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

observed by Hantschel et al.: the former mutant had an activity as low as that of the intact kinase, but the latter mutant had a kinase activity that was four-fold higher.

The whole linker (residues G2 to N80) between the myristoyl group and the SH3 domain of c-Abl has been modeled as flexible. There is some evidence that residues toward the C-terminal of the linker may have specific interactions with the SH3 domain. Hantschel et al. [2] found that the K70A mutation increased kinase activity by ca. six-fold. Screening for mutations on Brc-Abl leading to resistance of the kinase inhibitor drug STI-571 by Azam et al. [8] identified residues between K70 and N80. The scenario that residues K70 to N80 are rigidly bound to the SH3 domain can be easily accommodated in the current theory. The flexible linker would end at K70, hence the linker length 11 residues shorter. The distance between the myristoyl group and residue K70 would be required. Though this calculation cannot be carried out because K70 is not visible in the current structure of c-Abl, results qualitatively similar to those presented above are expected.

A major difference between bimolecular and intramolecular binding is that the latched fraction increases with ligand concentration in the former case but does not have a concentration dependence in the latter case. The bimolecular latched fraction equals the intramolecular one when the ligand concentration equals the effective concentration. Linking the myristoyl group to the kinase thus eliminates the need to maintain a high concentration ($\sim$0.5 mM according to Eq. 2) of the ligand in the cell. The myristoylated N-terminal of c-Abl is reminiscent of the N-terminal inactivation segment of the Shaker potassium channel [9,10]. There the main consideration is the binding kinetics – rapid inactivation of the ion channel is crucial for the modulation of the firing frequency of neurons [11]. For c-Abl, the requirement for a high latched fraction has been emphasized here. It is not clear whether the binding kinetics of the myristoyl group is of biological importance.

It may now be argued that a main function of the N-terminal sequence of c-Abl is to provide a tether with an appropriate length between the myristoyl latch and the rest of c-Abl. Incorporating existing experimental results into a theoretical model, the pathway for activating the c-Abl kinase through the dissociation of the myristoylated N-terminal latch is now quantitatively characterized. This should motivate quantitative studies of other pathways of activating c-Abl (e.g. displacement of the SH3 or SH2 domain).

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