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Structural Dynamic Studies of Matrix Metalloproteinase-9 Reveal New Details about its Activation Mechanism

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Malignant tumors express high levels of zinc-dependent endopeptidases called matrix metalloproteinases (MMPs). These metalloenzymes are thought to facilitate tumor metastasis and angiogenesis by hydrolyzing components of the extracellular matrix*(1)*. Thus, MMPs are an attractive target for development of antimetastatic drugs, aimed at inhibiting the pathologic activity of these enzymes. MMPs are produced in their inactive zymogenic forms (pro-MMPs), which are subsequently proteolytically activated in an elaborate set of events. The catalytic zinc ion in the pro-MMP is bound to three His and one Cys protein residues. Previous studies led to the formulation of the "cysteine switch hypothesis" as a model for understanding the unique structure of MMP zymogens and the means by which activation may be achieved *in vivo (2)*. Briefly, the cysteine switch model suggests that upon proteolytic activation, the latent zinc-binding site is converted to a catalytic zinc-binding site by dissociation of the thiol-bearing propeptide from the zinc atom and the binding of water *(3, 4)*. Yet the details of the catalytic mechanism of MMP enzymes, which leads to their activation, remain to be determined.

Using time-resolved X-ray absorption procedures developed in our laboratory (*5*) we have analyzed the structural and electronic changes occurring at the catalytic zinc environment of pro-MMP-9 during its activation. Figure 1 shows schematic representation of this experimental procedure, which allows to structurally characterizing catalytic sites of metalloenzymes in real time and in atomic resolution. We therefore, induced the activation reaction by rapidly mixing pro-MMP-9 with its activator protease and analyze the kinetic and the transient intermediate states that evolve at the catalytic site during this process. Surprisingly, we find that the catalytic zinc ion in pro-MMP-9 structurally rearranged prior to the dissociation of

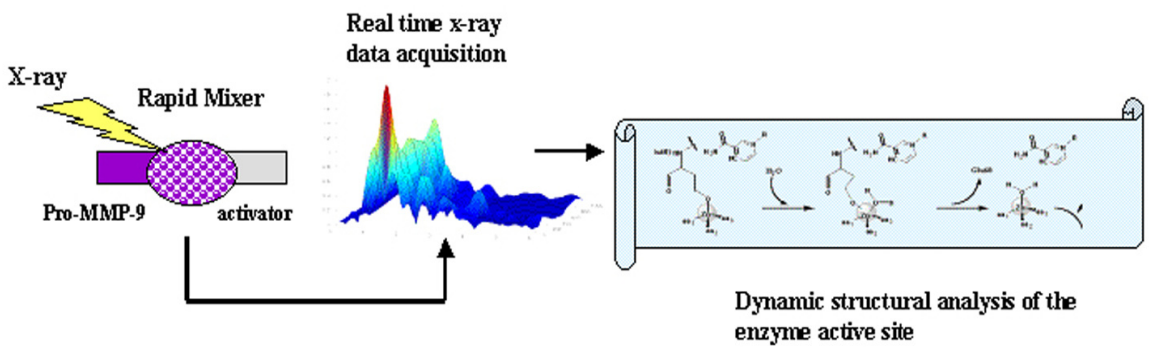

Fig.1 *Schematic representation of time-resolved X-ray absorption spectroscopy conjugated with transient kinetic analysis of metalloenzymes, recently developed in our laboratory (1). Briefly, we use stopped-flow apparatus to rapidly mix 1-4 reaction components. The reaction is followed by transient kinetics using optical spectroscopy and the structure of the catalytic metal site of the studied enzyme is probed by X-ray absorption spectroscopy. This procedure allows characterizing the structure of catalytic intermediates, which evolve during various enzymatic activities in real-time, and in atomic resolution. Importantly, this structural-dynamic analysis may be directly correlated with the distinct kinetic phases of the enzymatic reaction.*

the pro-polypeptide. Apparently these structural rearrangements are the consequence of long range conformational changes induced by the binding of the activator protease to the pro-peptide. These results suggest that the pro-domain in pro-MMP-9 is only "loosely bound" to the enzyme *in vivo*. This may allow the binding of small molecules such as inhibitors and cellular factors to the active site of the zymogen *in vivo*.

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