Inactivation of plasminogen activator inhibitor-1 by a small-molecule antagonist

Under a research project funded by the National Natural Science Foundation of China, Dr. Huang Mingdong, a professor of Fujian Institute of Research on the Structure of Matter, Chinese Academy of Sciences (CAS), and his colleagues published their research findings in *Chem Biol* (**2013**, 20(2):253—61).

Plasminogen activator inhibitor-1 (PAI-1) is the physiological inhibitor of tissue-type and urokinase-type plasminogen activators (tPA and uPA), thus an inhibitor of fibrinolysis. It is a potential therapeutic target in thrombosis and cancer. Several types of PAI-1 antagonist have been developed, but the structural basis for their action has remained largely unknown due to the challenges in the structural study of PAI-1, especially propensity of PAI-1 to form aggregates.

By screening a library of 1,600 natural small molecular compounds, developed by Prof. Lihong Hu of SIMM of CAS, a compound (Embelin) was identified to inhibit PAI-1 with an IC_{50} value of 1.6 μM. This compound shows *in vivo* activity on the inhibition of cell migration and blood clot formation. Interestingly, Embelin slows down the aggregation of PAI-1, allowing the crystallization of PAI-1:Embelin complex, and the subsequent structure determination at a resolution of 2.6 Å. The structural study (Figure 1) showed that Embelin is localized in a groove bordered by α helix D, helix F, β-strand 2A, and the loop connecting β-strand 1A to hE, close to the "flexible joints region" (α helix D and α helix E) and the "shutter region" (the central part of β-strand 5A and β-strand 3A and the underlying α helix B). Its polar ring sits in the middle of the groove forming direct strong contacts with Asp-95, Thr-93, Tyr-79, interacts with His-143, and has weaker interactions with Thr-94, and Met-45. The aliphatic chain of Embelin stretches out of the cavity and interacts with Ser-119 and Trp-139. To further validate the details of these interactions, the effects of five PAI-1 mutants, Y79A, T94A, D95A, S119A, and H143A, were studied by chromogenic assay to evaluate the inhibition of each mutant by Embelin. These site-directed mutagenesis results were indeed consistent with the determined crystal structure (Table 1). The authors further studied the mechanism of PAI-1 inhibition by Embelin (Figure 2). In the absence of Embelin, most of PAI-1 formed a covalent complex with its target protease (uPA) and thus inhibited uPA. When PAI-1 was pre-incubated with Embelin, the intensity of the complex band was reduced in a time-dependent manner. Thus, Embelin induces conversion of PAI-1 into a substrate of uPA.

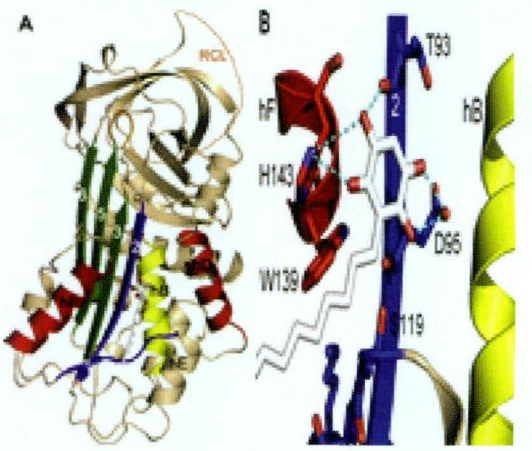

Figure 1

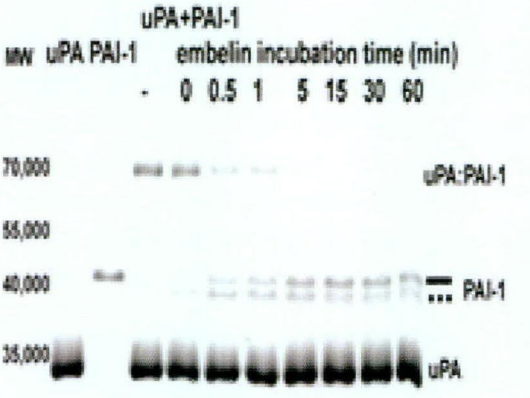

Figure 2

Table 1. The Effect of PAI-1 Mutation on the IC_{50} of Embelin

Mutants	IC_{50} (μM)
Wild-type	1.62 ± 0.16[a]
Y79A	3.15 ± 0.67
T94A	1.67 ± 0.15
D95A	15.2 ± 1.87
S119A	2.23 ± 0.38
H143A	12.0 ± 1.80

[a] IC_{50} mean value(μM) ± standard deviation, based on at least three independent experiments.

Table 1