Investigators perfect new version of blood-regulator thrombin

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In research led by a Saint Louis University investigator, molecular biologists have discovered a way to harness the enzyme thrombin's anti-blood clotting properties. The finding opens the door to new medications that will treat diseases related to thrombosis, the presence of blood clots in blood vessels, which is responsible for nearly a third of all deaths in the U.S.

"Thrombosis is one of the most prevalent causes of fatal disease," said lead researcher Enrico Di Cera, M.D., chair of the department of biochemistry and molecular biology at Saint Louis University School of Medicine. "If we could develop an anti-thrombotic drug that didn't carry a risk of hemorrhage, it would revolutionize the treatment of cardiovascular disease, the leading cause of death in the U.S. and Western world.

"This research carries us closer to that goal."

Blood clotting has long ensured our survival, stopping blood loss after an injury. On the other hand, if triggered in the wrong conditions, clotting can lead to debilitating or fatal conditions like heart attack, stroke and deep vein thrombosis.

Funded by the National Institutes of Health, and published in the June 18, 2010 edition of The Journal of Biological Chemistry (Vol. 285. No. 25), researchers zeroed in on thrombin, a vitamin K-dependent enzyme key to blood coagulation.

An unusual enzyme, thrombin performs distinct and even opposing functions, acting as a pro-coagulant, pro-thrombotic but also as an anti-coagulant factor depending on which target protein - fibrinogen, PAR1 or protein C - becomes activated in the blood. Researchers studied thrombin to decipher the structure-function code that enables this protein to do so many different things.

Tackling this problem far below the level of tissue and organs, molecular biologists looked deep inside the structure, examining thrombin's amino acids to note how they behave and interact with each other.

Using protein engineering, researchers produced mutations in the enzyme's amino acid sequence, carefully taking out pieces and replacing them, a few at a time, to find the exact locations that influence the function of thrombin. Once they found these "hot spots," researchers went even further - trying each of the 20 natural amino acids to see which mutation would allow them to turn on and off the pro-coagulant, pro-thrombotic and anti-coagulant functions.

"We asked the question, what if we can take this enzyme and dissociate the functions, allowing only the function we want?" said Di Cera.

In earlier research, Di Cera's team did just that. They engineered thrombin to promote activity toward protein C - the anticoagulant target protein - and minimize activity toward fibrinogen and PAR1 - the procoagulant and prothrombotic targets.

"In 2000, we engineered a thrombin mutant with potent anticoagulant properties both in vitro and in vivo and we are moving this mutant to a phase I trial," said Di Cera. "In this study, however, we pressed further. We wanted to optimize this mutant to completely abrogate activity toward fibrinogen and PAR1."

"With this research we optimized the mutant so that there is no clotting at all. Furthermore, we generated a new mutant with exclusive prothrombotic activity, thereby demonstrating that the individual functions of thrombin can be dissociated by replacing a single amino acid in the protein."

Once clinical trials are performed, researchers hope to have developed an alternative to heparin, a blood thinner that is used to prevent blood clots and

is often used before surgery, but which also causes allergic reactions, dosage challenges and bleeding.

"Heparin is a brute-force remedy that shuts down all thrombin functions, including its beneficial anti-coagulant role," said Di Cera. "Our approach is a new strategy that like a smart bomb only targets the functions we want to turn off."

Provided by Saint Louis University