Evolution provides clue to blood clotting

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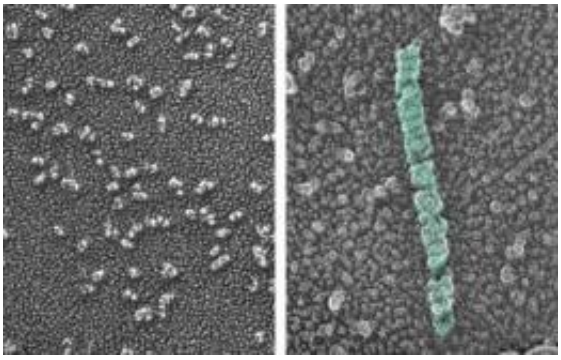

The building blocks of von Willebrand Factor remain separate at the slightly basic pH of 7.4 (left). In a more acidic environment (right), the VWF building blocks self-assemble into long chains and form the protein's signature helical tubules. This shape is vital to blood clotting. When VWF in the blood finds sites of injury, its helical tube unfurls to catch platelets and form blood clots. Credit: J. Evan Sadler, M.D., Ph.D.

A simple cut to the skin unleashes a complex cascade of chemistry to stem the flow of blood. Now, scientists at Washington University School of Medicine in St. Louis have used evolutionary clues to reveal how a key clotting protein assembles. The finding sheds new light on common bleeding disorders.

The long tube-shaped protein with a vital role in blood clotting is called von Willebrand Factor (VWF). Made in cells that form the inner lining of blood vessels, VWF circulates in the blood seeking out sites of injury. When it finds them, its helical tube unfurls to catch platelets and form

blood clots. Defects in VWF cause von Willebrand Disease, the most common inherited bleeding disorder in humans.

"The challenge for the cell is how to build this massive protein without clogging the machinery," says J. Evan Sadler, MD, PhD, professor of medicine and senior author of the study published in July in the Journal of Biological Chemistry. "The cell has solved this problem by making the assembly of von Willebrand Factor dependent on its location in the cell."

And VWF knows its location in a cell because pH, a measure of how acidic or basic a liquid is, varies from one cellular structure to the next. On a scale of 0 to 14, pure water has a neutral pH of about 7; human blood is slightly basic with a pH of 7.4.

In a cell, the building blocks of VWF form in an area with the same pH as blood. Then these building blocks are shipped to an area that is more acidic. Called the Golgi, this cellular compartment is known for its role in packaging proteins and has a pH of about 6.2. In this acidic environment, the building blocks of VWF are able to form long chains and fold into its signature helical tubules. But how this assembly process works has not been well understood.

From basic biophysics, Sadler and his colleagues knew that only one amino acid in the long protein chain is likely to "sense" a pH change from 7.4 to 6.2. Moving to an acidic environment, this amino acid, histidine, gains a positive charge. The group suspected that this charge may trigger the VWF building blocks to link together in a long chain.

But there are many histidines located throughout the chain. Like 26 letters of the alphabet form thousands of words, 20 essential amino acids form all proteins in the body. To identify which histidines might be guiding the amino acid chain to form the long VWF tubules, Sadler and

his team looked to evolution.

"If a particular histidine is important in this process, it should be present in the same location across many species," Sadler says.

So Sadler's group, including the paper's first author, Luke T. Dang, who was an undergraduate student when he did this work, gathered the DNA sequences of VWF for humans, 19 other placental mammals, a marsupial, two birds, a reptile, an amphibian and five fish. Dang is now a graduate student at the University of Washington, Seattle.

"By lining up the sequences, we found a relatively small number of histidines that are in the same place across species," Sadler says. "It then becomes manageable to mutate them individually and see if that prevents von Willebrand Factor from assembling."

Out of the many histidines in the amino acid sequence of VWF, they found two that are important in sensing the pH change and guiding the building blocks to form chains in an acidic environment. When Dang replaced either of these histidines with an amino acid that provides no positive charge, the chain did not form. But when Dang forced a positive charge to always be present at these locations, the chain formed again.

"A positive charge at these positions is important for von Willebrand Factor to assemble properly so it can perform its biological function," says Sadler, also a hematologist who specializes in treating patients with blood clotting disorders. "Without VWF, you bleed."

According to Sadler, defects in VWF disproportionately affect women because the protein is especially important for controlling bleeding during menstruation and childbirth. Sadler says this work helps to better understand the defects in pathways that cause von Willebrand Disease and related conditions.

More information: Dang LT, Purvis AR, Huang RH, Westfield LA, Sadler JE. Phylogenetic and functional analysis of histidine residues essential for pH-dependent multimerization of von Willebrand Factor. *Journal of Biological Chemistry*. July 2011.

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