Medical Xpress

Increasing the potency of HIV-battling proteins

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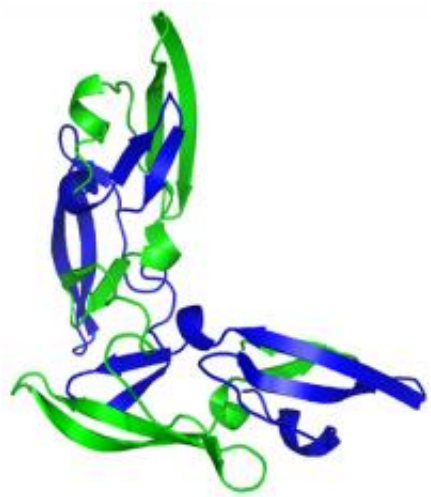

The experimentally determined structure of one of the engineered dimers (CVN2L0). One CV-N repeat is shown in green, while the other appears in blue. The polypeptide linker is not shown. Credit: Caltech/Jennifer Keeffe

If one is good, two can sometimes be better. Researchers at the California Institute of Technology (Caltech) have certainly found this to be the case when it comes to a small HIV-fighting protein.

The protein, called cyanovirin-N (CV-N), is produced by a type of blue-green algae and has gained attention for its ability to ward off several diseases caused by viruses, including HIV and influenza. Now Caltech researchers have found that a relatively simple engineering technique can boost the protein's battling prowess.

"By linking two cyanovirins, we were able to make significantly more potent HIV-fighting molecules," says Jennifer Keeffe, a staff scientist at Caltech and first author of a new paper describing the study in the Proceedings of the National Academy of Sciences (PNAS). "One of our linked molecules was 18 times more effective at preventing infection than the naturally occurring, single protein."

The team's linked pairs, or dimers, were able to neutralize all 33 subtypes of HIV that they were tested against. The researchers also found the most successful dimer to be similar or more potent than seven well-studied anti-HIV antibodies that are known to be broadly neutralizing.

CV-N binds well to certain carbohydrates, such as the kind found in high quantities connected to the proteins on the envelope that surrounds the HIV virus. Once attached, CV-N prevents a virus from infecting cells, although the mechanism by which it accomplishes this is not well understood.

What is known is that each CV-N protein has two binding sites where it can bind to a carbohydrate and that both sites are needed to neutralize HIV.

Once the Caltech researchers had linked two CV-Ns together, they wanted to know if the enhanced ability of their engineered dimers to ward off HIV was related to the availability of additional binding sites. So they engineered another version of the dimers—this time with one or more of the binding sites knocked out—and tested their ability to neutralize HIV.

It turns out that the dimers' infection-fighting potency increased with each additional binding site—three sites are better than two, and four are better than three. The advantages seemed to stop at four sites, however;

the researchers did not see additional improvements when they linked three or four CV-N molecules together to create molecules with six to eight binding sites.

Although CV-N has a naturally occurring dimeric form, it isn't stable at physiological temperatures, and thus mainly exists in single-copy form. To create dimers that would be stable under such conditions, the researchers covalently bound together two CV-N molecules in a head-to-tail fashion, using flexible polypeptide linkers of varying lengths.

Interestingly, by stabilizing the dimers and locking them into a particular configuration, it seems that the group created proteins with distances between binding sites that are very similar to those between the carbohydrate binding sites in a broadly neutralizing anti-HIV antibody.

"It is possible that we have created a dimer that has its carbohydrate binding sites optimally positioned to block infection," says Stephen Mayo, Bren Professor of Biology and Chemistry, chair of the Division of Biology, and corresponding author of the new paper.

Because it is active against multiple disease-causing viruses, including multiple strains of HIV, CV-N holds unique promise for development as a drug therapy. Other research groups have already started investigating its potential application in prophylactic gels and suppositories.

"Our hope is that those who are working to make prophylactic treatments using cyanovirin will see our results and will use CVN_2L0 instead of naturally occurring cyanovirin," Keeffe says. "It has higher potency and may be more protective."

The paper, entitled "Designed oligomers of cyanovirin-N show enhanced HIV neutralization," was published in the online edition of PNAS. In addition to Keeffe and Mayo, other authors on the paper include

research technician Priyanthi N.P. Gnanapragasam, former biology graduate student Sarah K. Gillespie, biology graduate student John Yong, and Pamela J. Bjorkman, the Max Delbruck Professor of Biology at Caltech and a Howard Hughes Medical Institute investigator.

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