NIST suggests new purity test for biotech products

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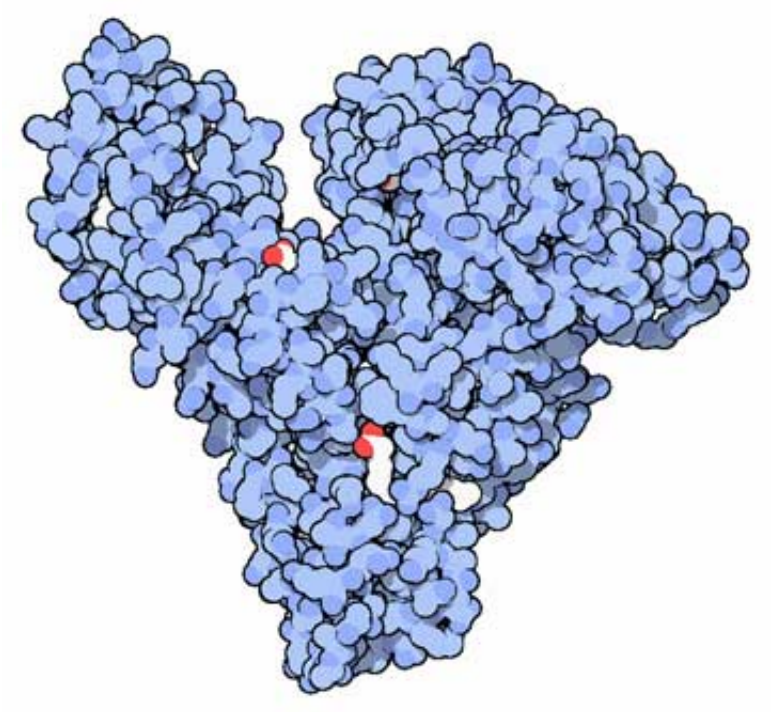

Human serum albumin (HSA) manufactured by genetically engineered seeds is commonly used for a variety of biomedical applications, from cell-growth media to clinical uses, but a new study suggests that plant-derived HSA may be contaminated by low levels of plant enzymes that could affect its properties. Credit: David S. Goodsell, RCSB PDB

To avoid contaminating their experiments, biomedical researchers want to know that the scientific products they buy are pure. But how pure does something need to be to really be pure? Using a new test, scientists

at the Institute for Bioscience and Biotechnology Research (IBBR) have found traces of plant enzymes in batches of supposedly pure, commercially available human blood protein genetically manufactured from plant seeds. Because they are active agents that promote biochemical reactions, enzyme contamination at even low levels could have an outsized effect on measurement reproducibility, and quality control in biomanufacturing.

The IBBR is a joint institute of the National Institute of Standards and Technology (NIST) and the University of Maryland.

The enzyme concentrations found in the study are so low that standard quality control tests cannot detect them. But even very small amounts of the catalytic proteins could cause spurious results that render experiments confusing or meaningless. And in a clinical context, if the enzymes end up in blood products given to patients, they might cause toxic or immune reactions.

The contamination discovery came about by chance, while IBBR chemists Robert Brinson and John Marino and their colleagues were studying how different molecules bind to a protein called human serum albumin (HSA). HSA makes up around three to five percent of our blood and is an important carrier for natural substances as well as drugs in the bloodstream. HSA also has proven useful as a component of media for growing cell cultures, which are used to manufacture proteins for many biotechnology and clinical purposes. Thanks to recombinant DNA technology, HSA no longer has to come from humans. To mass-produce the protein at low cost, scientists have engineered the gene that codes for it into rice seed. Rice-derived HSA is now available from a number of vendors.

In their study, the team used a technique called nuclear magnetic resonance, or NMR, to look for particular types of chemical bonds

between commercially available HSA and other molecules. But the scientists saw something unexpected in their NMR results. Bonds between phosphate groups and carbon atoms were breaking in one of the tested molecules, adenosine triphosphate, or ATP. Few enzymes can break such bonds, and because the researchers were studying a plant-derived product, they suspected one culprit in particular: a group of plant enzymes called phytases.

Apparently, the phytases had eluded manufacturers' quality control tests. These tests, which can include the commonly used enzyme-linked immunosorbent assay (ELISA) as well as a combination of liquid chromatography and mass spectrometry, are targeted to specific contaminants. That is, you have to know what you're looking for to find it. And even though these tests can detect impurities down to around 1 part per billion, enzymes can "have an outsized effect" even at very low concentrations, Marino says, because, like chemical catalysts, they accelerate biochemical reactions without being consumed in the process.

Because their background effect might raise or lower concentrations of key biomolecules, phytase contamination could cause researchers to misinterpret experimental results based on cell cultures grown in HSA-based medium, Brinson and Marino say. And while they did not test any drug products, they note that HSA-based therapies often come in relatively large doses, making even slight contamination a potentially serious health risk.

After their initial discovery, the team tested seven HSA products from four different vendors and found varying levels of phytase activity in each batch. They also tested human serum-derived and yeast-derived HSA products but did not detect carbon-phosphate bonds breaking, providing further evidence for contamination by plant enzymes in the other products.

Brinson and Marino say that NMR screening could provide an important quality control test for the increasing number of plant-produced biotechnology products. Human proteins, vaccines and other biological substances are now produced in corn, tobacco and alfalfa, as well as rice. In all cases, manufacturers need to ensure that enzymes like phytases do not end up in the finished product, and NMR could be useful for this process, Marino says. "It's a very useful assay specifically tailored to plant production of these proteins."

But the researchers emphasize that their method is an addition to, not a replacement for, traditional screening methods. "What we're trying to propose is that our NMR methods will directly complement what's already being used," Brinson says.

More information: R.G. Brinson, G.G. Giulian, Z. Kelman, J.P. Marino. "Detection of contaminating enzymatic activity in plant-derived recombinant biotechnology products." *Analytical Chemistry*. Published online Nov. 13, 2014. DOI: 10.1021/ac503864m.

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