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Title: Reactions of Urate Hydroperoxide with Biological Targets

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Introduction:

Do you like beer, red meat, and seafood? If you do, you need to know about a big problem that these delicious foods can pose for you. When your body breaks them down, they produce a lot of uric acid. Normally uric acid dissolves in our blood and is readily excreted in our urine. However, when you eat too much meat and seafood, or drink too much beer, uric acid may precipitate and form sharp crystals your joints – particularly in your big toe. This condition is known as gout. High uric acid levels in blood are also associated with cardiovascular disease and diabetes. I have spent my summer working on the chemical reactions uric acid can undergo when it is at high concentrations in the blood. I have investigated the formation of urate hydroperoxide - a molecule thought to be produced during the body's inflammatory response. This occurs during inflammation when urate reacts with superoxide - a chemical produced by inflammatory cells. Little is known about urate hydroperoxide and its role in the body so this project aimed to provide insight into the reactions of urate hydroperoxide. Ultimately, we want to know whether it acts as a signal to tell the body there is inflammation, is a reactive molecule that kills pathogens, or a toxin that promotes disease.

Aim:

The main aims in this project were to show that two enzymes, lactoperoxidase (LPO) and xanthine oxidase (XO), work together to form urate hydroperoxide, and that this product could react with proteins and become attached to them.

Method:

I synthesised urate hydroperoxide with LPO and XO. In most experiments, I monitored formation of urate hydroperoxide using a spectrophotometer or by a chemical test known as the FOX assay. This assay was used to show that the formation of urate hydroperoxide required superoxide and the product was indeed a hydroperoxide. I also used mass spectrometry to confirm that urate hydroperoxide was produced by the enzymes. Preliminary experiments were conducted to determine the time required to form the maximal amount of product, and whether the addition of vitamin C would slow or stop its production.

Results:

Experiments showed that formation of urate hydroperoxide was superoxide-dependent. This was proven by the full system producing a peak in the UV-visible spectra at 315 nm that was not present in the controls. The FOX assay was used to show that LPO and XO use urate to produce a superoxide-dependent hydroperoxide and mass spectrometry confirmed that this superoxide-dependent hydroperoxide was urate hydroperoxide. A preliminary experiment indicated that vitamin C significantly slowed/stopped the formation of the product. This finding, however, was not taken any further.

Conclusion:

The formation of urate hydroperoxide by LPO and XO could be an important cellular process used during an inflammatory disease. Urate hydroperoxide may form attachments on proteins in the blood. These attachments could be measured and could indicate the level of inflammatory disease in the body.

The next step for this project is to work out why LPO and XO work together to create urate hydroperoxide. Does urate hydroperoxide act as a signal to tell the body there is inflammation? Is it a reactive molecule that can attack infecting microorganisms or is it toxic to our own cells?