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Project: Inactivation of enzymes by urate hydroperoxide and protection by ascorbic acid

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Introduction:
Uric acid, or urate, is the product of normal breakdown of energy molecules in the body. Eating foods such as oily fish, alcohol and red meat raise the levels of urate in our bloodstream. High urate levels are linked with inflammatory diseases such as gout, kidney failure and cardiovascular disease.

Clearly urate has toxic potential when at a high concentration during inflammation. We wanted to understand the underlying molecular mechanisms of this link. During inflammation, white blood cells called neutrophils degrade urate to the reactive molecule urate hydroperoxide. Urate hydroperoxide may damage important gears in cells. For example, urate hydroperoxide could damage biologically active proteins called enzymes. The attachment of urate to enzymes may affect their ability to function and cause tissue damage to occur. This theory could explains urates contribution to inflammatory diseases.

Aim:
The aim of this project was to determine whether urate hydroperoxide permanently binds to small segments of proteins called peptides.

Method:
Urate hydroperoxide was produced by the enzymes lactoperoxidase and xanthine oxidase. Urate hydroperoxide and the peptides MLTELEK or TNFFEK were mixed together in tubes to see if a product was formed. A highly sensitive technique called mass spectrometry was used determine the molecular weight of peptides. An increase in molecular weight with urate hydroperoxide treatment would indicate attachment of urate to the peptide.

Results:
We found that urate hydroperoxide added 140 Daltons to the N-terminal of the peptides. For MLTELEK and TNFFEK the urate became attached to the lysine (K). We made a series of control samples where urate hydroperoxide was not made and there was no evidence of a urate attachment. We could not determine the exact structure of the urate attachment.

Conclusion:
We have preliminary data to show that urate could bind proteins. However, the exact identity of the urate attachment is unclear as the molecular weights do not add up. The increase in molecular weight of the peptides was 140 Daltons, whereas the molecular weight of urate is 168 Daltons. Urate may break apart during the reaction to result in a lower molecular weight.

Further research is required to determine the chemical structure of urate attachment. My results show that urate could attach to biologically active proteins during inflammation. The scope of this project did not allow me to assess the protective ability of ascorbate but this will be addressed in the future.

The next step is to test blood samples from patients with gout for presence of the urate attachment to proteins. This would prove this is a physiological phenomenon and adds to our understanding of how high urate levels in our blood could cause inflammatory disease.