

Automated LC-MS/MS Data Analysis of a Novel Alcohol Dehydrogenase Form Isolated from Pigeon Liver

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In the transition from genomics to proteomics, there is an increased need for automated protein analysis. Huge data sets from LC-MS/MS analyses are becoming common place and automated data processing has become crucial. In this project, we have purified a previously uncharacterised major ethanol-active alcohol dehydrogenase form (K_m for ethanol in the low mM range) from pigeon livers and characterised it using LC-MS/MS. All generated data has been processed automatically.

The purified enzyme was subjected to a number of proteolytic enzymes with varying specificity. The separate digest mixtures were injected into a nano-LC system with a 15cm x 75 μ m reversed phase column. This was coupled on-line with a hybrid quadrupole orthogonal acceleration time-of-flight tandem mass spectrometer, Q-TOF Ultima API, operated in data dependent acquisition mode. MS/MS data were subsequently analyzed by a tiered series of algorithms. After the initial pass against a database, the unannotated MS/MS spectra are passed through to an algorithm that searches for possible modifications, including amino acid substitutions, against the identified protein sequences. The remaining spectra, of sufficient quality, were then automatically *de novo* sequenced. The resulting protein sequence was then assembled using the overlapping peptide sequences, generated from the different enzymatic specificities, to enable a single consensus protein sequence to be obtained.

This process unexpectedly established a novel enzyme form of a class normally exhibiting a high K_m for ethanol. This occurrence of one further class-mixed ADH form helps elucidate the process of enzymogenesis and shows that the vertebrate alcohol dehydrogenase system is an excellent model system for several stages of successive changes.