

Detection of Protein Isoaspartate: a Ubiquitous Post-Translational Modification

Joshua Alfaro, Laura Gillies, He Sun, Shujia Dai, Tianzhu Zang, Joshua Klaene, Byung Ju Kim, Jonathan Lowenson,

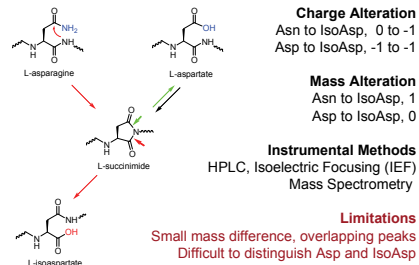
Kathy Dorgan, Steven Clarke, Barry Karger, and Zhaohui Sunny Zhou*

Barnett Institute and Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115

Isoaspartate and Succinimide Formation and Significance

Isoaspartate (isoAsp) formation is a ubiquitous post-translational modification (PTM) arising from spontaneous asparagine (Asn) deamidation or aspartate (Asp) isomerization via succinimide intermediates. When isoAsp forms, a methylene group is inserted into the protein backbone, generating a "kink", and thus may drastically alter protein structure and function, and in most cases has been implicated with protein damage. On the other hand, it also plays a role in signaling and regulation, such as a molecular clock in aging. Moreover, significant isoAsp accumulation is commonly observed in a myriad of protein reagents and pharmaceuticals, affecting their efficacy and toxicity. Hence, there is growing interest in characterizing isoAsp in both biological research and protein pharmaceutical development.

Isoaspartate and Succinimide Formation and Current Detection Methods



Our Chemo-Enzymatic Approach

In our approach, the labile isoaspartyl methyl esters and succinimides are trapped with nucleophilic hydrazines or hydroxylamines to form hydrazides or hydroxamic acids, respectively. The peptidyl hydrazides are stable and can be directly detected and sequenced using standard liquid chromatography and mass spectrometry. Moreover, several options are available to facilitate characterization. For instance, hydrazides can be affinity-enriched with aldehyde resins, offering the potential for large scale analysis of complex samples. Additionally, stable isotope tags can be introduced for relative quantification or facile sequence determination. Furthermore, hydrazides can be selectively derivatized with various chromogenic or fluorogenic aldehydes or sulfonyl chlorides tags, allowing identification and absolute quantification without mass spectrometers.

Hydrazide Chemistry for Dummies

Esters are electrophilic.

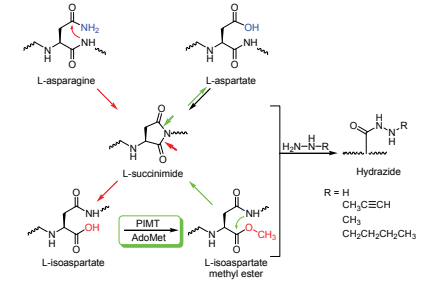
Water is not an organic solvent!

1st Ed., Z.S.Z. Press, 1999, Ann Arbor, Michigan.

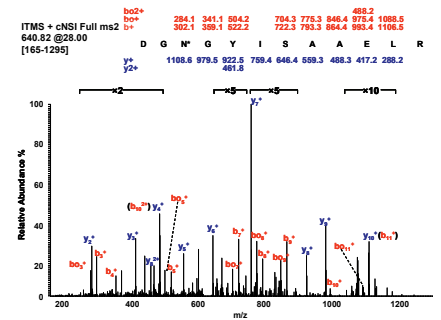
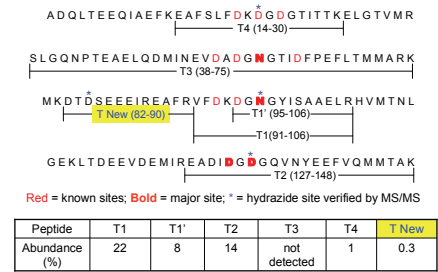
Enzymatic Method and Limitations

A much higher degree of specificity toward isoAsp is achieved through an enzymatic method using protein-isoaspartate O-methyltransferase (PIMT or PCMT, EC 2.1.1.77), a process commercialized by Promega under the name IsoQuant. The PIMT enzyme methylates isoAsp to the corresponding methyl ester, using S-adenosyl-methionine (AdoMet or SAM). As a result, the presence and location of isoAsp can be deduced from the isoaspartyl methyl esters. However, labile isoaspartyl methyl esters spontaneously cyclize to succinimides, which then hydrolyze to isoAsp or Asp, formation of the latter restores the native protein backbone as part of the protein damage repair pathway. Since methyl ester cleavage can be rapid with half lives as short as 4 min, the intrinsically labile methyl ester functionality is not a reliable tag for detection.

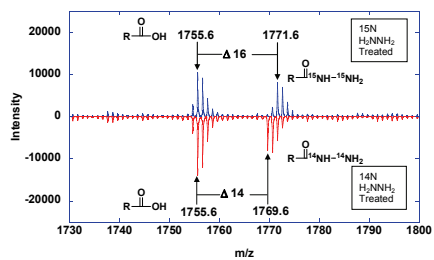
Hydrazinolysis of Ester and Succinimide



Identify Hydrazides in Calmodulin

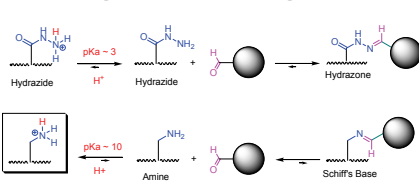


Stable Isotope Labeling of Hydrazides



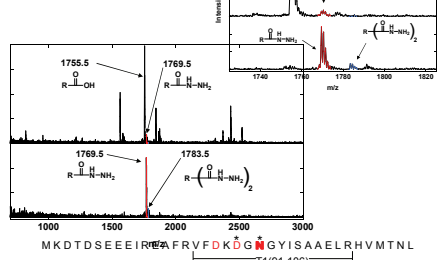
Do I have to memorize the pKa values?

Affinity Enrichment of Hydrazides



At pH 3-6, selectivity > 10,000

Affinity-Enrichment of Hydrazides



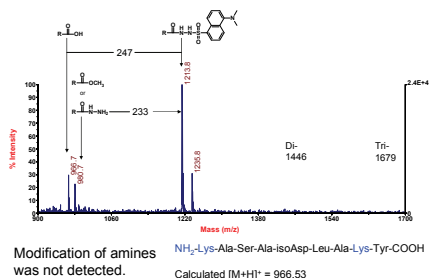
Think Big, Start Small.

pH = 5

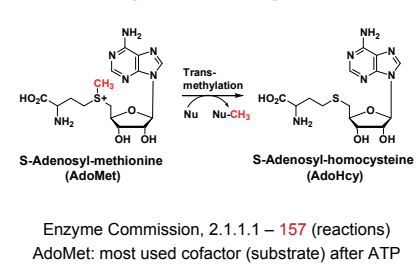
pH < 1

pmole scale
2 μ L aldehyde beads

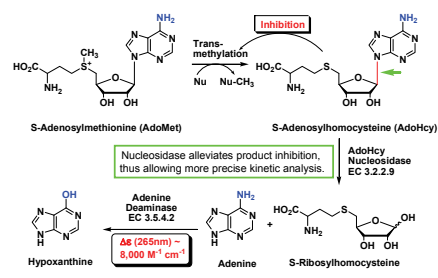
Hydrazide Modification with Dansyl Chloride



AdoMet-Dependent Methyltransferases



Continuous Spectrometric MTase Assay



Protein MTase Assay

