

Hydrogen–deuterium exchange in imidazole as a tool for studying histidine phosphorylation.

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Streszczenie

Isotope exchange at the histidine C2 atom of imidazole in D2O solution is well known to occur at a significantly slower rate than the exchange of amide protons. Analysis of the kinetics of this isotope-exchange reaction is proposed herein as a method of detecting histidine phosphorylation. This modification of His-containing peptides is challenging to pinpoint because of its instability under acidic conditions as well as during CID-MS analysis. In this work, we investigated the effect of phosphorylation of the histidine side chain in peptides on deuterium–hydrogen exchange (DHX) in the imidazole. The results demonstrate that phosphorylation dramatically slows the rate of the DHX reaction. This phenomenon can be applied to detect phosphorylation of peptides at the histidine residue (e.g., in enzymatic digests). We also found that the influence of the peptide sequence on the exchange kinetics is relatively small. A CID fragmentation experiment revealed that there was no detectable hydrogen scrambling in peptides deuterated at C2 of the imidazole ring. Therefore, MS/MS can be used to directly identify the locations of deuterium ions incorporated into peptides containing multiple histidine moieties.

Słowa kluczowe

Phosphohistidine, Hydrogen–deuterium exchange (HDX) reaction, Deuterium–hydrogen exchange (DHX) reaction, Mass spectrometry

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