

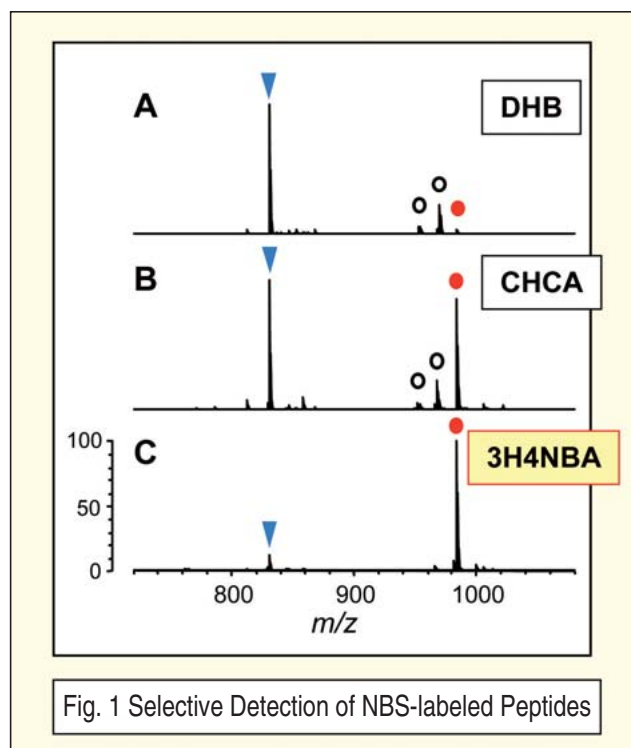
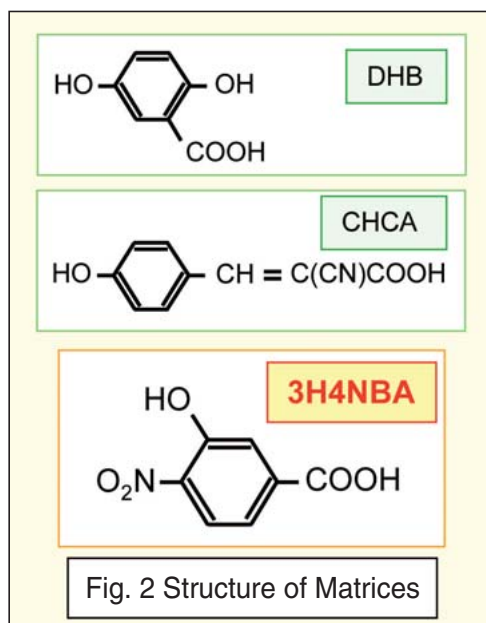
Selective Ionization, Detection by MALDI-TOF MS with NBS-labeled Peptides using New Matrix

Previously, we developed a new quantitative proteome analysis method (NBS protocol) based on stable isotope labeling.¹⁾ Here we report that use of 3H4NBA (3-hydroxy-4-nitrobenzoic acid; Fig. 2) as the matrix for MALDI-TOF MS analysis of NBS reagent (¹²C₆-NBS or ¹³C₆-NBS; 2-nitrobenzenesulfonyl chloride) labeled peptides results in the selective detection of the labeled compounds (Fig. 1).

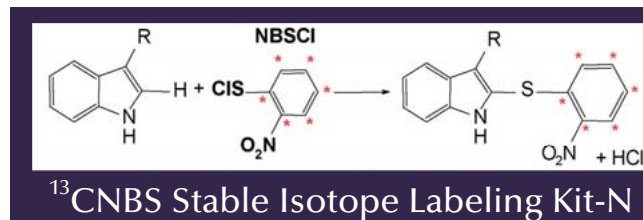
In addition, when the 3H4NBA matrix is used, the generation of neutral loss peaks (○) often observed when analyzing complex peptide mixtures is suppressed.

● = NBS-labeled peptides
▼ = Non-labeled peptides

Even though the samples measured are identical (NBS-labeled peptides : non-labeled peptides = 1:1), there is clearly a large, matrix-dependent, difference in the analysis results. Identifying a matrix that selectively ionizes NBS-labeled peptides greatly widens the NBS methods potential applications and utility.

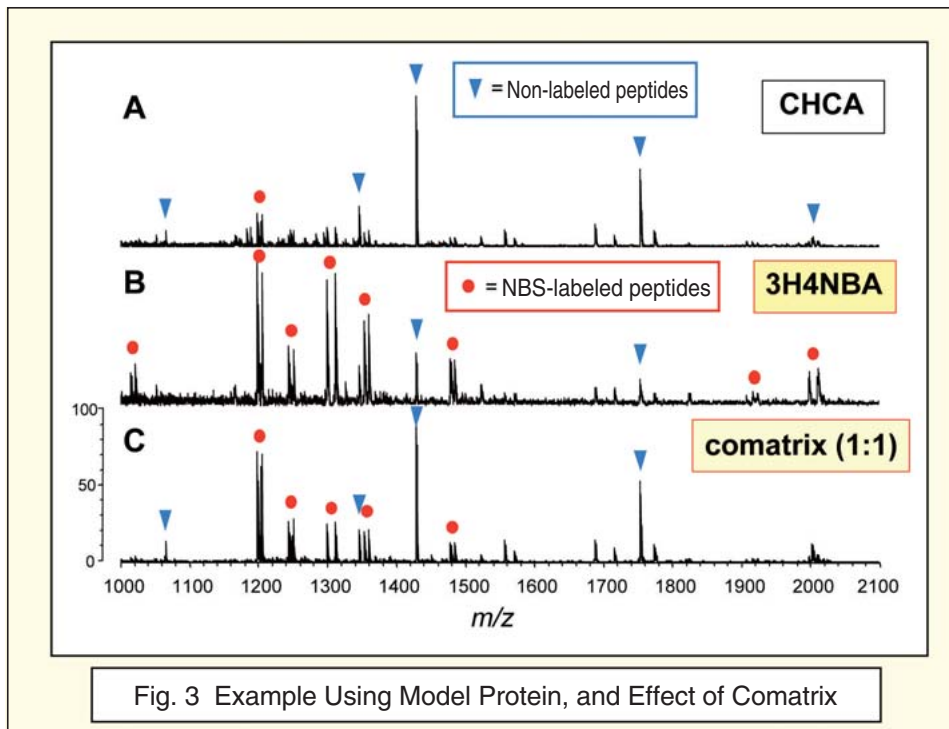


While attempting to identify a chemical rationale for the 3H4NBA matrix's selectivity, it was found to be important that both the (selectively) detected substrate and matrix possess a nitrobenzene skeleton²⁾.

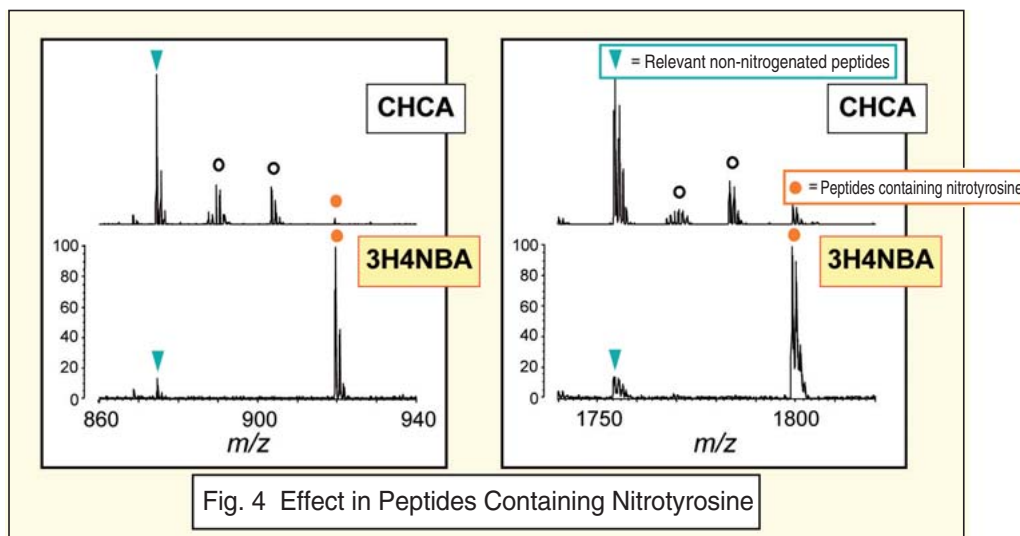


* For details on NBS method, refer to AXIMA Application No. 12 and 16.

Next, an example of processing a model protein according to the NBS protocol is shown. Utilizing the selectivity of the 3H4NBA matrix, it is evident that the labeled pair peaks (●) were preferentially detected (Fig. 3A, B). It was also found that by using a mixture of 3H4NBA and CHCA (co-matrix), versus each of them individually, the sensitivity (S/N ratio) observed was approximately 4 times greater. (Fig. 3C)



It appears that the 3H4NBA selective matrix effect can also be applied to other compounds containing nitrobenzene. In the next example, upon investigating the effect with respect to one of the most common biological compounds containing a nitro group, nitrotyrosine, it is evident that the same selectivity effect is displayed (Fig. 4).



References

- 1) H. Kuyama et al., *Rapid Commun. Mass Spectrom.* **17**, 1642-1650 (2003)
- 2) E. Matsuo et al., *Proteomics* **6**, 2042-2049 (2006)



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