Quantitative proteome analysis by labeling tryptophan residues (NBS Method)

The stable isotope reagent labeling method for performing global quantitative analysis of expressed proteins in samples of 2 different states (e.g., pathological and normal models, etc.), is excellent in compared with existing methods with respect to quantification and reproducibility, and is therefore receiving much attention.

The method introduced here uses stable isotope labeling (NBS method) (Fig. 1) onto tryptophan residue in proteins and peptides for performing relative quantification by mass spectrometry. Fig. 1 shows the structure of the NBS reagent, 2-nitrobenzenesulfenyl chloride. NBS-heavy reagent incorporates six atoms of ^{13}C, a stable isotope of carbon, and the NBS-light reagent incorporates six ^{12}C atoms. Both NBS (Heavy) and NBS (Light) are chemically identical molecules, but their mass numbers differ by 6 Da. This difference in mass number permits relative quantitative analysis by comparing the peak areas in the mass spectrum of the peptides labeled with NBS (Heavy) and NBS (Light).
The protocol is outlined in Figure 2.

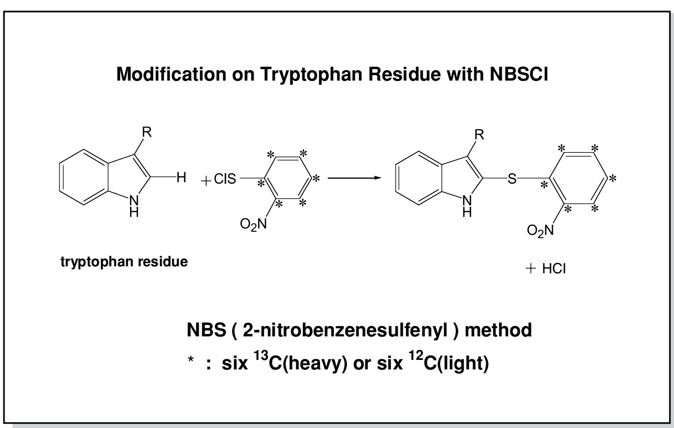


Figure 1 NBS Reagent

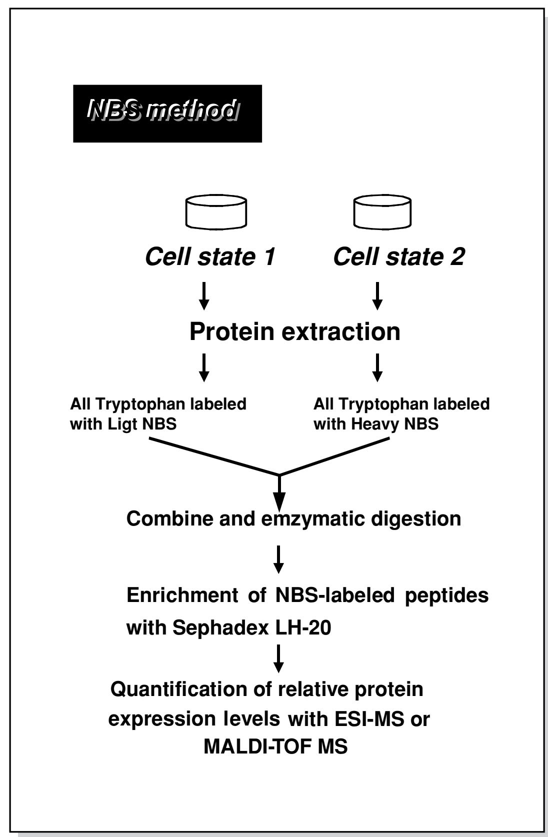


Figure 2 NBS Method Protocol Outline

The ICAT™ method has been known for one of stable isotope dilution techniqus which involves labeling on cysteine residue in proteins / peptides, concentrating the target labeled peptides using an affinity column, and then performing relative quantification using mass spectrometry. A major difference between our approach and that based on the ICAT™ strategy is that the former focuses on the tryptophan residue, and another difference is the use of Sephadex (LH-20) media for the separation of tryptophan-containing peptides after labeling. Tryptophan is the least abundant in a protein and widely distributed residues in proteins (over 90%). And it is the most hydrophobic amino acid residues, playing an important role in biological systems. The rarity promises simplicity in mass spectrometric data and separability with Sephadex media makes this method versatile.
The NBS approach offers a widely applicable means of analyzing protein mixtures derived from biological samples, and the method described here presents an effective and simplified approach to proteome analysis.

Figure 3 shows an example of relative quantification using model peptides (ACTH and galanin). The proportions of the labeled peptides in the samples were obtained according to the theoretical values (ACTH 1:1, galanin 1:2). Figure 4 shows the analytical results obtained after enrichment using Sephadex LH20 in the procedure shown in Figure 2, using a sample consisting of rat serum (normal). Figure 4(a) shows the spectrum prior to enrichment, Figure 4(b) post enrichment, and Figure 4(c) shows an enlargement of 4(b) (m/z: 700 - 1100). Many doublet peaks are seen in 4(c) (indicated by arrows) after enrichment column operation, and each of the peak pairs shows the relative abundance ratio (in this case, 1:1) of the expressed proteins. Figure 5 shows part of the results of relative quantitation of the expressed proteins in rat serum (normal and hyperglycemic) using ESI-MS. Several peak pairs (m/z: 756.5, 762.5; 826.5, 832.5; 883.5, 889.5) can be seen in Figure 5(a), and the doublet peak (m/z: 826.5, 832.5), which derived from albumin, was used to determine the relative abundance to be 1:1, as shown in Figure 5(b).

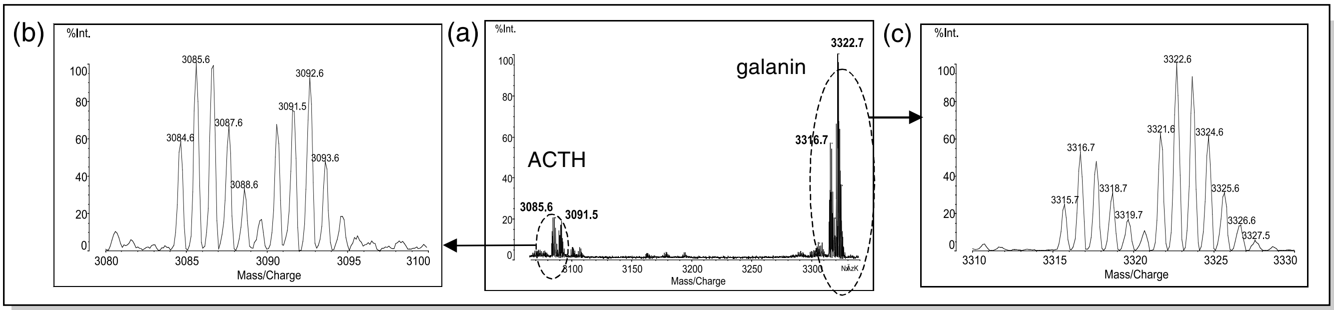
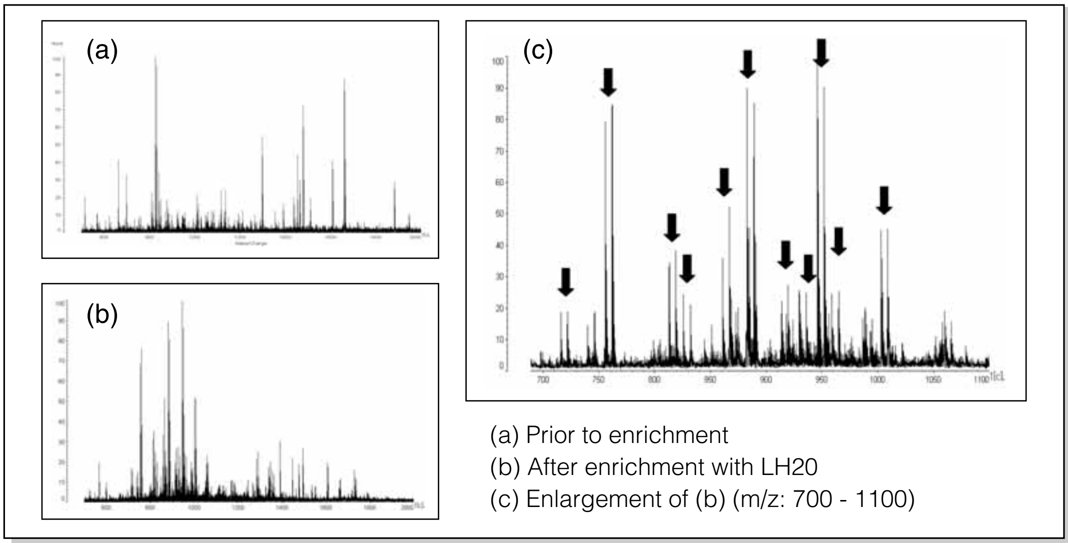


Figure 3 Relative Quantification with use of Model Peptides



(a) Prior to enrichment
(b) After enrichment with LH20
(c) Enlargement of (b) (m/z: 700 - 1100)

Figure 4 Enrichment of Tryptophan Processed with Sephadex LH20

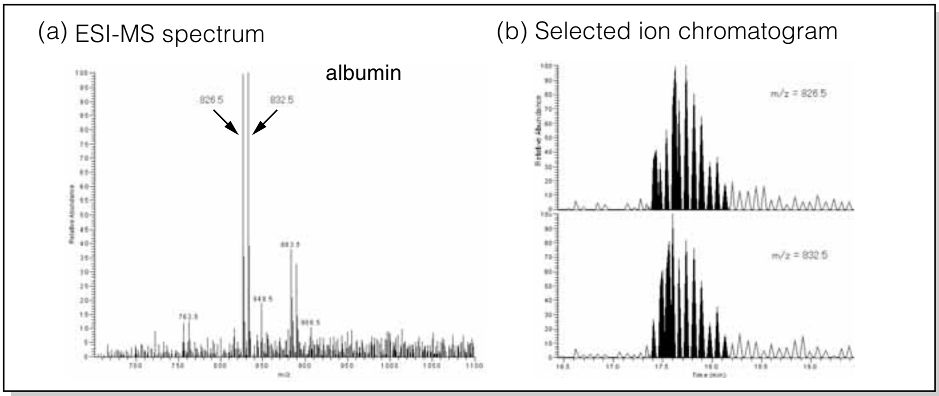


Figure 5 Relative Quantification of Expressed Proteins in Rat Serum (Normal and Hyperglycemic)

References: H.Kuyama, M.Watanabe, C.Toda, E.Ando, K.Tanaka, and O.Nishimura,
" An approach to quantitative proteome analysis by labeling tryptophan residues"
Rapid Commun. Mass Spectrom., 2003;17:1642-1650.

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