Improved Decipherment of the Protein Database of Human Proteins in the PDMD (Protein-Direct-Microsequencing-Deciphering) method

Abbreviations; EDC: 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide; SEC:Size-exclusion chromatography; NCBI: National Center for Biotechnology Information; BLAST: Basic Local Align Search Tool; ProtParam-Expathy: Protein parameter calculation tool. PDMD: Protein-Direct-Microsequencing-Deciphering.

Abstract

Human proteins seem to be processed by Human serum biotinidase, and Human excreted proteins seem to be handled with by Human serum biotinidase and Human Chymotrypsin A. Therefore, we must improve PDMD method by using these new findings. Protein determination is performed by the highly sensitive HPLC-SEC-photometric method at UV 210 nm; i.e., c.a. 200-fold sensitive than Lowry's method. Human proteins are found to be not metabolized at membrane inserted portions. Membrane and Hydrophobic proteins of Humans are defined as the precipitable proteins at 100,000 x g for 90 min at 4 C, and have hydrophobicities larger than 0.515

Key words; Microsequencing, Edman degradation, KPLC-with photometric detection, Proteomics, Protein determination

Introduction

We have previously reported the PDMD (Protein-Direct-Microseqencing-Deciphering) method, which is a uniquely quantitative method (1).

We also recognized that Human serum biotinidase has a unique amidase/peptidase, which can hydrolyze between Hydrophobic amino acid and Hydrophic amino acis or Hydrophilic amino acid (2). We also found that Humans also have Chymotrypsin A in Human pancreatic juice (3). Human pancreatic juice also has an amidase/esterase Lipoamidase/BSSL (4). We have found that Human lipoamidase excretion increases c.a. 100,000-fold in the Pancretic Cancer (our unpublished observation).

Human proteins seem to be processed by Human serum biotinidase, and Human excreted proteins seem to be handled with by Human serum biotinidase and Human Chymotrypsin A. Therefore, we must improve PDMD method by using these new findings.

Materials and Methods

Protein determination is performed by the highly sensitive HPLC-SEC-photometric method at UV 210 nm; i.e., c.a. 200-fold sensitive than Lowry's method (5). Proteins were appropriately diluted to 1 mg/mL, and were directly bound to Glass-fiber disc by using EDC (6). Microsequencing was performed by utilizing the PPSQ-21A protein sequencer (Shimadzu, Kyoto, Japan). Hydrophobicity of protein was calculated by utilizing ProtParam-Expathy. PDMD method was performed as previously described (1).

Hydrophobicity was calculated as follows; i.e., 1st; Sum of hydrophobic amino acids was calculated (Cys + Met) x 2 + Gly/2 + Ala + Ile + Leu + Phe + Pro + Tyr + Trp + Val (%). 2nd; sum (%)/100 was induced. Hydrophobicity larger than 0.515 was defined as the Hydrophobic protein.

Results

Human proteins seem to be processed by the Law of processing as follows; i.e.,
The method for determination of the presence or the absence of proteins and peptides in Humans is depended on the substrate specificity of Human Serum Biotinidase; i.e., Human Serum Biotinidase can not hydrolyze or metabolize those N-terminal structures, [1] XP- , [2] pyrE- and pyrD- , [3] D- , [4] X-D-Amino acids- such as X-D-Ala- , [5]

Acetyl-X-/AcX- and Formyl-X-/fX- (X is Ala, Leu, Met, Asp, and Lys), [6] Molecules which have internal or intra Cys-Cys bonds (within 6 position from N-terminal) such as Insulin and Avidin, [7] Molecules which have N- or O-glycochain within 6 position from N-terminus, and [8] Molecules which have UbI and/or SUMO at any positions

Human proteins are found to be not metabolized at membrane inserted portions.

Membrane and Hydrophobic proteins of Humans are defined as the precipitable proteins at 100,000 x g for 90 min at 4 C, and have hydrophobicities larger than 0.515.

Conclusion

Human proteins are processed by the rule of substrate specificity of Human serum Biotinidase. Thus, we surely improved PDMD method.

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