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

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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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.

Keywords: Microsequencing; Edman degradation; HPLC-with photometric detection; proteomics; protein determination.

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.*

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1. INTRODUCTION

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

2. MATERIALS AND METHODS

We also recognized that Human serum biotinidase has a unique amidase/peptidase, which can hydrolyze between Hydrophobic amino acids, and between Hydrophobic amino acid and Hydrophilic amino acid [1]. We also found that Humans also have Chymotrypsin A in Human pancreatic juice [2]. Human pancreatic juice also has an amidase/esterase Lipoamidase/BSSL (Bile-salt stimulated lipase) [3]. We have found that Human lipoamidase excretion increases c.a. 100,000-fold in the Pancreatic Cancer (our unpublished observation). Further, we also have found that Human kidney biotinidase excretion into the patient urine of Diabetes mellitus patients is elevated [4].

Human proteins seem to be processed by the Human serum biotinidase (our unpublished observation), and Human excreted proteins seem to be handled with by Human serum biotinidase (our unpublidhed observation) and Human Chymotrypsin A [5]. 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., HPLC SEC method is c.a. 200-fold sensitive than Lowry's photometric method [6]. Protein determination of proteins via the RP-HPLC-photometric method is also possible to be performed [7]. Protein determination can be done by this high-recovery method. Proteins loaded must be washed out from the ODS column by repeating the washing the ODS column by repeated Gradient elutions for c.a. 6-times (our unpublished observation).

The proteins were appropriately diluted to 1 mg/mL, and were directly bound to Glass-fiber disc by using EDC [8]. Microsequencing was performed by utilizing the PPSQ-21A protein sequencer (Shimadzu, Kyoto, Japan).

Hydrophobicity of protein was calculated by utilizing ProtParam-Expathy. Expasy was created in August 1993. Originally, it was called ExPASy (Expert Protein Analysis System) and acted as a proteomics server to analyze protein sequences and structures and two-dimensional gel electrophoresis (2-D Page electrophoresis).

PDMD method was performed as previously described [9].

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 (our unpublished observation). Hydrophobicity larger than 0.515 was defined as the Hydrophobic protein. Hydrophobicity of glycoproteins is amended by reducing 0.015 per Glycochain.

3. RESULTS AND DISCUSSION

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 [10], and (8) Molecules which have UbI and/or SUMO at any positions [11].

Human proteins are found to be not metabolized at membrane inserted portions (our unpublished observation).

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 amended hydrophobicities larger than 0.515. This knowledge of membrane glycoproteins is contributed to the improved PDMD method of membrane glycoproteins.

4. 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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COMPETING INTERESTS

Author has declared that no competing interests exist.

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