LC-MS^Eによるアイソフォームとホモログの同定と定量

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INTRODUCTION

通常、タンパク質の定性および定量解析は公開データベースを使って行われてい 通常、タンパワ質の定せあよび定量新術は公開データペースを使って行われている。 る。しかし、公開データペースは生体内に存在するタンパワ質の効果を非反映しているわけではない。タンパウ質は生体内ではプロセンングや翻訳後修飾などの変化を 受けているためである。これらの変化のうち、修動と変異の一部はデータペースに反映されており、務新にも用いられている。しかし、サーチアリニリズムには、与えられた 理論的な配列情報と質量分析器の測定結果から、より高い配列カバー率とより正 確な定量結果を提供することが期待されている。

そこでわれわれは、定性面では、in silico processingを用い、データベースに 生体内の配列を反映する情報を書き加える方法を採用した。また、定量面ではホ モログの存在するタンパク質を解析する際には、特異的なペプチドの平均イオン強度 を利用して、共通するペプチドのイオン強度をそれぞれのホモログに配分するという方 法を採用した。

このように改良された定量方法を、非常に高い配列相同性を持った14-3-3タン パク質ファミリーの絶対量および相対量の決定に使用したところ、生体内の量比を反 映した結果を得ることができた。

さらにヒト血漿中に存在する複数の鎖から構成されるタンパク質 Complement conc_immxでに付せまる数数の場所次で180ラハジ員 Complement C4 の解析を行った。in sillco processing を行うことですータハースに築情発展的 な情報を書き加え、そのデータバースを用いて解析を行った結果、各々の鎖がより簡 便に同定、定量されることが示された。

最後に、微生物システムにおいてタンパク質のアイソフォームの経時変化を定量した 例を示した。

METHODS

Sample preparation

Sample preparation The investigated samples included human blood platelets, a human NK2 cell line, undepleted human plasma and *Escherichia coli* grown on various carbon sources. In all linstances, the samples were denatured in the presence of 0.1% ApplGest at 80°C for 30 min. Next, the samples were centrifuged, the supernatants collected and the proteic concentration estimated by the Bradford method. Total protein extracts were subsequently reduced (10 mM DTT), alkylated (10 mM IAA) and enzymei:protein ratio for 16 hours at 37°C, then 1:50 for 4 hours at 37°C] of TPCK-treated trypsin.

LC-MS conditions

LC-MS conditions A single dimension LC-MS approach has been used for the separation and analysis of the samples. The combined qualitative and quantitative experiments were conducted using a 90 min gradient from 5 to 40% accontritie (0.1% formic acid) at 250 nL/min using a nanoACQUITY system. A BEH 1.7 µm C18 reversed phase 75 µm x 15 cm nanoscale LC column was used and estimated on-column sample loads were 0.5 µg protein digest for all studies. The data independent, alternate scanning LC-MS⁶ experiments were performed with a Synapt MS mass spectrometer.

In silico processina

Databases searches were conducted with dedicated search Databases searches were conducted with dedicated search algorithms against *in-silico* processed — Figure 1 — protein databases that were appended with all known annotated chains, the resolved signal, transit and propep features or only fully matured forms of the proteins. Additionally, initiating methionines were removed from all non-truncated proteins. Sequence unique information, often the C and N termin, can then be utilized for qualitative and quantitative purposes.

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RESULTS

Quantification principle

The quantitative aspect of the method utilizes the average intensities of unique peptides to apportion the total ion intensity to each parent protein homologue. The segmentation of unique and no unique peptides to each protein is shown in Figure 2.

The principle is demonstrated for the 14-3-3 protein family of The principle is demonstrated for the 14-3-3 protein family of which the amino add sequence is highly conserved — see Figure 3. Highlighted are sequence unique, proteotypic peptides identified to two human samples that can be utilized to parse the intensity for subsequent quantification. The corresponding fragment ion spectra are shown in Figure 4.

Table 1 shows the estimate concentration, illustrating the accuracy of the method and the determined stoichiometry for the proteins of interest. The average stoichiometry values are similar for both samples, suggesting equimolar flux.



14-3-3 タンパク質ファミリーのアミノ酸配列比較 灰色:複数のホモログタンパク質に共通する トリプシン消化ペプチド

indant nentides NK2 cells 1433B_HUMAN: QTTVSN SQQAYQI







14-3-3タンパク質ファミリーのタンパク質特異的ペプチドおよび 共通ペプチドの同定結果



NK2細胞と血小板中の14-3-3タンパク質ファミリーの ラベルフリー定量と化学量論解析

Chain annotation and quantitative analysis

Protein chains can only be quantified if chain specific information is populated in the protein sequence database. An example of a well annotated database entry is shown in Figure 5.

This information can be utilized by both the search and the quantification algorithm to estimate the concentration of the various circulating chains, unlike presenting a group value for the complete group of proteins — see Figure 6.



Figure 5. Complement C4のタンパク鎖と断片情報



Complement C4のα鎖/γ鎖およびβ鎖/γ鎖のヒト血漿中の Figure 6. モル比 (n=3) それぞれ2:4および2:5に近い量比となっている

Microbial systems

Despite the fact that microbial systems have near complete genomes, certain microbial proteins exhibit sequence similarity due to preservation. Fumurate dehydrogenases are notorious examples where approximately 90% of the sequence is preserved — top pane Figure 7.

The most abundant peptide GVYNTYIEDNLR is sequence The most abundant peptide GVMTTLEDNLK is sequence common — middle pane Figure 7 — and intensity parsing is therefore required to provide more accurate relative and absolute quantification measurement and stoichiometry figures and trends for *E. coli* grown on various carbon sources — bottom pane. The latter allows the determination of the regulation of the B form.



ラクトース培地および酢酸塩培地で培養したE.coliにおける Figure 7. Fumurate dehydrogenaseのアイソフォームA、Bの 相対および絶対発現量

CONCLUSION

In silico processingと組み合わせることでタンパク質特異的な配列データに 基づいたにC-MS⁵を用いたラベルフリー定量はアイソフォームおよびホモログタンパク 質の相対えた10絶対定量、化学量論解析、パスウェイ解析とタンパク頃含量の決 定に有効であることが示された。

References

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