Theoretical ribosomal protein mass distribution of *Pseudomonas aeruginosa* PAO1

Wenfa Ng

Unaffiliated researcher, Singapore, Email: ngwenfa771@hotmail.com

Abstract

Ribosomes are the protein synthesis factories of a cell and thus are evolutionary conserved in structure and function. Comprising a large and small subunit, the ribosome is further made up of ribosomal proteins that give structure and function to different parts of the macromolecular complex. Current methods for isolating the ribosome include density gradient ultracentrifugation that separates the ribosome into the large and small subunit. Separation of the various ribosomal proteins that comprise each of the subunit would require a solubilization step followed by the use of sodium dodecyl sulphate and polyacrylamide gel electrophoresis (SDS-PAGE). However, possibility exists for the use of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to profile the set of ribosomal proteins that could be solubilized from each ribosome subunit. Using ribosomal protein amino acid sequence information from Kyoto Encyclopaedia of Genes and Genomes (KEGG), the molecular weight of each ribosomal protein from *Pseudomonas aeruginosa* PAO1 was calculated in this report. Obtained results revealed that each ribosomal protein had a unique mass that could be detected by mid-range MALDI-TOF MS instruments. More importantly, the mass of ribosomal proteins constitutes a unique mass fingerprint of each ribosome subunit, which accounts for the different structure and functions of the large and small ribosome subunit. Overall, current mass resolution of MALDI-TOF MS instruments could resolve ribosomal proteins and thus provides a tool for profiling the set of ribosomal proteins that constitute the large and small subunit of the ribosome.

Keywords: ribosomal proteins, large subunit, small subunit, ribosome, MALDI-TOF MS, mass resolution, mass spectrometry,

Subject areas: biochemistry, microbiology, bioengineering, biotechnology, bioinformatics,

Highlights

1) Calculation of *Pseudomonas aeruginosa* PAO1's ribosomal protein molecular mass from amino acid sequence information reveals the mass of individual ribosomal protein could be differentiated from others by modern mid-range MALDI-TOF mass spectrometers.

2) Unique ribosomal protein mass fingerprint exists for ribosomal proteins of the large and small ribosome subunit.

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Introduction

Ribosome consists of a large and small subunit, which together is responsible for protein synthesis in the cell. Ribosomal proteins constitute both the large and small subunit, and provide structure and function to the ribosome. Thus, given the importance of ribosome to cellular function, its structure should be highly conserved across the three domains of life, where differences between ribosomes from species in different domains arises from divergence in evolutionary paths. Such differences in ribosome structure could be attributed to differences in structure and function of the constituent ribosomal proteins.

Isolation of both ribosome subunits could be achieved with density gradient ultracentrifugation where the prokaryotic ribosome fractionates into a 30S small subunit and 50S large subunit. Additional biochemical fractionation of each subunit would subsequently yield a set of ribosomal proteins of each subunit. Current methods for determining the set of ribosomal proteins that constitute the small and large ribosome subunit rely on solubilization of the ribosome subunit followed by the use of sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (SDS-PAGE). Detection of proteins could be achieved by either mass spectrometry or chemiluminescence methods. Specifically, mass spectrometry methods such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) or electrospray ionization tandem mass spectrometry (ESI MS/MS) could provide the identity of the proteins after bioinformatic analysis.[1 2] Chemiluminescence detection, on the other hand, would rely on the use of specific antibodies for ribosomal protein identification.

Using a curated resource of ribosomal protein amino acid sequence information of *Pseudomonas aeruginosa* PAO1, this study tests the feasibility of the alternative approach of profiling the ensemble of ribosomal proteins in both the large and small ribosome subunit. Specifically, the approach seeks the use of MALDI-TOF MS for profiling the constituent ribosomal proteins in the large and small ribosome subunit after solubilization of the ribosomal proteins from each subunit. Doing so requires distinct mass differences between individual ribosomal proteins resolvable by modern mid-range MALDI-TOF MS which has mass resolution of at least 1 Da. Hence, the objective of this study is to construct a theoretical mass fingerprint of ribosomal proteins in the large and small ribosome subunit for understanding if the criterion of resolvable mass differences between individual ribosomal proteins is fulfilled in the case of *P. aeruginosa* PAO1 ribosomal proteins.

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Materials and methods

Construction of theoretical ribosomal protein mass distribution of Pseudomonas aeruginosa PAO1

Ribosomal proteins amino acid sequence information was obtained from Kyoto Encyclopaedia of Genes and Genomes (KEGG) database for *Pseudomonas aeruginosa* PAO1 (http://www.genome.jp/dbget-bin/www_bget?pae03010). Molecular weight of the proteins was calculated using the Compute pI/Mw tool at (https://web.expasy.org/compute_pi/).

Results and Discussion

Table 1: Theoretical molecular weight of ribosomal proteins in the small ribosome subunit of *P. aeruginosa* PAO1

Protein name	Molecular weight (Da)
rpsA; 30S ribosomal protein S1	61869.86
rpsB; 30S ribosomal protein S2	27336.42
rpsC; 30S ribosomal protein S3	25838.06
rpsD; 30S ribosomal protein S4	23277.58
rpsE; 30S ribosomal protein S5	17625.38
rpsF; 30S ribosomal protein S6	16164.66
rpsG; 30S ribosomal protein S7	17504.42
rpsH; 30S ribosomal protein S8	14170.6
rpsI; 30S ribosomal protein S9	14614.79
rpsJ; 30S ribosomal protein S10	11766.6
rpsK; 30S ribosomal protein S11	13629.65
rpsL; 30S ribosomal protein S12	13799
rpsM; 30S ribosomal protein S13	13265.41
rpsN; 30S ribosomal protein S14	11565.43
rpsO; 30S ribosomal protein S15	10115.62
rpsP; 30S ribosomal protein S16	9204.45
rpsQ; 30S ribosomal protein S17	10085.78
rpsR; 30S ribosomal protein S18	8873.35
rpsS; 30S ribosomal protein S19	10357.26
rpsT; 30S ribosomal protein S20	9917.59
rpsU; 30S ribosomal protein S21	8484.9

Table 1 shows the molecular weight of various ribosomal proteins of the small ribosome subunit in *P. aeruginosa* PAO1. The unique mass of each ribosomal protein in the subunit suggests

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the existence of a unique mass fingerprint of ribosomal proteins of a ribosome subunit. Specifically, isolation of the specific subunit followed by mass spectrometry profiling of ribosomal proteins would provide a mass fingerprint. On the other hand, profiling of ribosomal proteins by SDS-PAGE and comparison of molecular weight of separated protein bands with the molecular weight ladder could not deliver a unique mass fingerprint at 1 Da mass resolution given inherent limitation of the molecular weight ladder's mass resolution. Thus, mass spectrometry profiling of ribosomal proteins by MALDI-TOF MS enables the determination of the unique ribosomal protein mass fingerprint of individual ribosome subunit.

Table 2: Theoretical molecular weight of ribosomal proteins of the large ribosome subunit of *P. aeruginosa* PAO1

Protein	Molecular weight (Da)
rplA; 50S ribosomal protein L1	24234.08
rplB; 50S ribosomal protein L2	29579.07
rplC; 50S ribosomal protein L3	22591.67
rplD; 50S ribosomal protein L4	21639.91
rplE; 50S ribosomal protein L5	20391.79
rplF; 50S ribosomal protein L6	19099.13
rplL; 50S ribosomal protein L7/L12	12479.39
rplI; 50S ribosomal protein L9	15531.72
rplJ; 50S ribosomal protein L10	17634.31
rplK; 50S ribosomal protein L11	14907.16
rplM; 50S ribosomal protein L13	16028.53
rplN; 50S ribosomal protein L14	13411.86
rplO; 50S ribosomal protein L15	15174.42
rplP; 50S ribosomal protein L16	15401.15
rplQ; 50S ribosomal protein L17	14503.82
rplR; 50S ribosomal protein L18	12661.61
rplS; 50S ribosomal protein L19	13032.21
rplT; 50S ribosomal protein L20	13364.89
rplU; 50S ribosomal protein L21	11653.58
rplV; 50S ribosomal protein L22	11910.94
rplW; 50S ribosomal protein L23	10949.7
rplX; 50S ribosomal protein L24	11470.45
50S ribosomal protein L25/general stress protein Ctc	21962.24
rpmA; 50S ribosomal protein L27	8990.4
rpmB; 50S ribosomal protein L28	9065.56
rpmC; 50S ribosomal protein L29	7201.34
rpmD; 50S ribosomal protein L30	6477.62

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50S ribosomal protein L31 type B	9518.61
rpmE; 50S ribosomal protein L31	7920.17
rpmF; 50S ribosomal protein L32	6791.52
rpmG; 50S ribosomal protein L33	6045.1
rpmH; 50S ribosomal protein L34	5209.23
rpmI; 50S ribosomal protein L35	7363.88
50S ribosomal protein L36	5975.34
rpmJ; 50S ribosomal protein L36	4434.38

Similarly, Table 2 shows the theoretical molecular weight of ribosomal proteins in the large ribosome subunit of *P. aeruginosa* PAO1. Given that each ribosomal protein has a unique molecular weight, a unique mass fingerprint exists for the ribosomal proteins in the large ribosome subunit of *P. aeruginosa* PAO1. Thus, unique ribosomal protein mass fingerprint exists for each ribosome subunit, and this help explain the unique structure and function of each subunit. Specifically, presence of different sets of ribosomal proteins in each subunit accounts for differences in structure and function of the large and small ribosome subunit.

Conclusions

Calculated theoretical molecular weight of ribosomal proteins yield a unique mass fingerprint for ribosomal proteins of the large and small ribosome subunit of *P. aeruginosa* PAO1. This suggests the use of MALDI-TOF MS tool for profiling the set of ribosomal proteins from each ribosome subunit after solubilization of proteins from the subunit. Higher mass resolution of MALDI-TOF MS over conventional SDS-PAGE would provide a richer dataset useful for subsequent identification of ribosomal proteins through comparison with a proteome database. Overall, MALDI-TOF MS could provide accurate determination of ribosomal protein mass distribution in each ribosome subunit given the presence of unique mass of each ribosomal protein. The mass spectrometry approach also offers an easier and faster method for profiling the set of ribosomal proteins in each ribosome subunit compared to gel electrophoresis and antibody based identification methods. Existence of unique mass fingerprints of ribosomal proteins in the large and small ribosome subunit also illuminates another layer of biological significance. Specifically, differences in the structure and function of the large and small ribosome subunit arose from different sets of ribosomal proteins of different molecular mass in each subunit.

Supplementary materials

Raw data of theoretical calculation of ribosomal protein's molecular mass is appended as an Excel file.

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Conflicts of interest

The author declares no conflicts of interest.

Author's contribution

The author conceived the idea, did the calculation, analysed the data, and wrote the manuscript.

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