【Grant-in-Aid for Scientific Research(S)】
Science and Engineering (Chemistry)

Title of Project：Quantitative Analyses of Transcription and Translation Processes on a QCM

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Research Area：Bio-related Chemistry

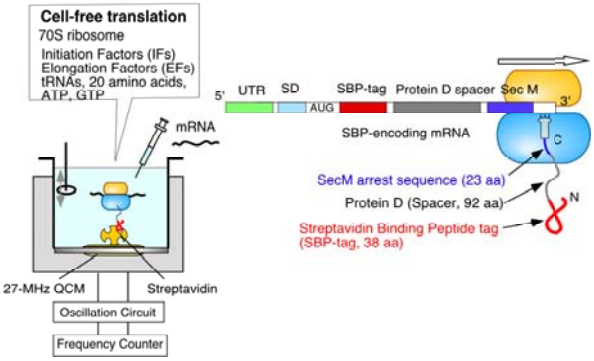
Keyword：Quarzt-crystal microbalance, Transcription, Translation, Mass changes

【Purpose and Background of the Research】

Proteins are synthesized by ribosomes, which decode the genetic information within mRNAs that have been transcribed from DNA. Many factors interact with the ribosome during protein synthesis, even in bacteria, to enable the sophisticated reactions of translation to occur. The translation process in bacteria is divided into three steps. Firstly, a bacterial ribosome binds to the Shine-Dalgarno (SD) sequence on an mRNA to form an initiation complex (translation initiation). Secondly, the ribosome catalyzes the polymerization of amino acids to form a polypeptide chain. Thirdly, the completed polypeptide chain is released and the ribosome is recycled (translation termination). To evaluate the translational efficiency of a specific mRNA from the formation of the initiation complex until translation termination, a series of single-turnover translation reactions must be observed in real time. Here we plan the real-time monitoring of a cell-free and single-turnover translation reaction on a 27-MHz quartz-crystal microbalance (QCM) with no labeling.

【Research Methods】

To investigate the reactions of protein translation, we established a system that allowed the real-time monitoring of protein synthesis using a cell-free translation mixture and a 27-MHz quartz-crystal microbalance (QCM). Using an mRNA that encoded a fusion polypeptide comprising the streptavidin-binding peptide (SBP tag), a portion of Protein D as a spacer, and the SecM arrest sequence, we could follow the binding of the SBP tag, while it was displayed on the 70S ribosome, to a streptavidin-modified QCM over time. Thus, we could follow a single turnover of protein synthesis as a change in mass. This approach allowed us to evaluate the effects of different antibiotics and mRNA sequences on the different steps of translation. From the results of this study, we determined that both the formation of the initiation complex from the 70S ribosome, mRNA and fMet-tRNAfMet, or the accommodation of the second aminoacyl-tRNA to the initiation complex is rate-limiting steps in protein synthesis.



【Expected Research Achievements and Scientific Significance】

We are planning the novel application of the QCM technique to the observation of a single-turnover reaction of protein synthesis in real time with no requirement for labeling. The combination of QCM and a reconstituted cell-free translation system permitted the detection of the newly synthesized protein with a quite high sensitivity, and the evaluation of different antibiotics and analysis of the mechanism of translational regulation by the 5'UTR. Using this methodology, the effects of sequences such as UTRs and ORFs on protein synthesis and protein folding can be analyzed by simply measuring a change in mass.

【Publications Relevant to the Project】

1) S. Takahashi, R. Akita, H. Matsuno, H. Furusawa, Y. Shimizu, T. Ueda, and Y . Okahata, *ChemBioChem*, 9, 870-873 (2008).
2) S. Takahashi, M.Iida, H. Furusawa, Y. Shimizu, T. Ueda, and Y. Okahata, *J. Am. Chem. Soc.*, 131, 9326-9332 (2009).

【Term of Project】 FY2010-2014

【Budget Allocation】 142,200 Thousand Yen

【Homepage Address】
http://www.okahata-lab.bio.titech.ac.jp/