The pH dependence of peptide release from the ribosome

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Protein synthesis or translation is one of the core processes in the cell. The genetic information stored in DNA is first transcribed into mRNA and then translated into proteins that participate in nearly all processes in the cell and ensure its functioning. Proteins are synthesized by ribosomes – cellular machines that quickly and accurately translate the sequence of nucleotides (codons) in mRNA into a sequence of amino acids in proteins. Stop codons in mRNAs signal the end of the protein. These codons are recognized by protein release factors (RF) that induce the release of the finished protein from the ribosome. This project is about how and at what rate the RFs induce the cleavage of the bond holding the protein on the ribosome. By studying pH dependence of the release rate we can obtain useful information about the reaction mechanism of this cleavage.

Ribosomes can function even without cells – they can synthesize proteins in a test tube (in vitro) if all necessary components are added to the reaction mix. In our experiments we used a synthetic mRNA encoding a protein consisting of three amino acids followed by a stop codon. These mRNAs are translated by ribosomes in vitro but the finished protein can not be released until release factors are added to the reaction mix. In this way we can start the release reaction by adding RFs only when we are ready to follow its course. For this purpose we used two fast kinetics methods, quench flow and stopped flow, which can measure the rate of very fast reactions lasting less than few seconds. In this way we could determine the maximal rate (k_{cat}) of peptide release at different pH values in the reaction mixture.

Our results show that the rate of peptide release is very sensitive to pH and increases with the increasing concentration of hydroxide ions in solution. We have also observed a saturation of the reaction rate at high pH that probably indicates a conformational change the RF has to undergo to become active. Such changes have been reported from protein crystallography studies and were suggested to occur when a stop codon is recognized. We also propose a reaction mechanism that could explain the pH dependence of the rate of release we observed in our experiments.

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