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CONTRIBUTION OF HYDROPHOBIC AMINO ACIDS TO THE STABILITY AND ACTIVITY OF
AN ANCESTRAL PROTEIN**Abstract**

Modern organisms uses 20 amino acid set to synthesize a protein and this feature is derived from our last common ancestor (LUCA). However, earlier protein synthesis was plausibly much simpler than modern one and utilized less than 20 amino acid set. We used one of the resurrected nucleoside diphosphate kinases, named Arc1 which is already devoid of cysteine and consists of 19 amino acids. Also, unfolding midpoint temperature of Arc1 is 114 and their specific activity is significant. Therefore, we eliminated hydrophobic amino acids (Alanine, Phenylalanine, Isoleucine, Leucine, Methionine) from Arc1 to confirm the necessity of 20 amino acids for earlier protein synthesis. We eliminated each hydrophobic amino acid from Arc1 and constructed five simplified Arc1 variants and it showed thermal stability and catalytic activity. We also simultaneously removed two of Alanine, Phenylalanine, Isoleucine, Leucine and Methionine, but all variants folded stable and catalytically active structure. We further eliminated three of Alanine, Phenylalanine, Isoleucine, Leucine and Methionine together and gained the variant, which was devoid of Phenylalanine, Isoleucine and Methionine and comprised of 16 amino acids, demonstrating substantial specific activity. Finally, we excluded Alanine, Phenylalanine, Isoleucine and Methionine at once, but this variant was insoluble. In conclusion, we obtained the variant which consists of 16 amino acids and this result suggest that it is not necessary for synthesizing stable and catalytically active proteins to utilize 20 kinds of amino acids. However, we can't affirm it only by these results, so we are going to do further amino acid exclusion experiments.