Observation on the Interaction of a Valyl-Adenylate-Synthetase Complex with Its Transfer Ribonucleic Acid and the Implication Thereof

JUDITH O. WILLIAMS

Departments of Microbiology, and Physiology and Biophysics, University of Illinois, Urbana, Illinois 61803

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The charging of a transfer ribonucleic acid (tRNA) can be written as a two-step reaction: (i) amino acid + adenosine triphosphate (ATP) + enzyme \(\rightarrow\) aminoacyl-adenosine monophosphate (AMP)-enzyme + pyrophosphate; (ii) aminoacyl-AMP-enzyme + tRNA \(\rightarrow\) aminoacyl-tRNA + enzyme + AMP. The aminoacyl-AMP-enzyme complex (E\(_a\)) formed in the first reaction has been isolated and shown to react with tRNA to yield, as expected, aminoacyl tRNA. This has been done in a number of systems: the isoleucine system of Escherichia coli (2, 5); a valine system of yeast (3, 4); a threonine system of rat liver (1); and leucine and valine systems of E. coli (M. Kondo and C. Woese, in preparation).

All of these investigations report that only 50 to 70% of the amino acid in the form of E\(_a\) can be transferred to tRNA, even though tRNAs are in great excess and reaction times are, for practical purposes, infinite. This result, however, cannot be attributed in all cases to hydrolysis of the E\(_a\). For example, Norris and Berg showed that untransferred isoleucine remains reactive with hydroxylamine. We also have observed a similar incomplete transfer of valine to its tRNA from the valyl-E\(_a\) in a system from E. coli (Fig. 1). In other experiments, the extent of transfer could not be increased by increasing the incubation time or the tRNA concentration.

However, when the tRNA charging by E\(_a\) is done under conditions in which separation of certain of the components of the reaction mixture occurs, then one can observe 100% transfer of the E\(_a\)-bound amino acid to tRNA, as the following experiment shows. \(^{14}C\)-valyl-E\(_a\), isolated by gel filtration, was chromatographed on a Sephadex G100 column that had been previously equilibrated with SS buffer (see Fig. 1, legend) containing uncharged (total) tRNA at a concentration of 20 \(\mu\)g/ml. The same supplemented buffer was used as the eluant. E\(_a\) is not retarded by this gel, whereas the tRNAs are retarded. Under these conditions, all of the \(^{14}C\)-valine is transferred to tRNA (Fig. 2). Free valine or valyl-AMP resulting from any breakdown of the

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1 Present address: Department of Chemistry, Washington State University, Pullman, Wash. 99163.
Eₐ would have eluted between fractions 10 and 15 and would have been detected. The broadness of the peak indicates that the transfer took place gradually as the complex passed through the column. Approximately 6 hr elapsed between loading and elution of the enzyme.

Under the conditions where transfer of amino acid to tRNA is incomplete (i.e., where reactants and products remain intermixed), the incomplete transfer is probably not caused by an equilibrium which favors aminoacyl-AMP-containing compounds, because no strong dependence of the extent of reaction upon initial Eₐ concentration is seen even when tRNA is present in great excess. (The number of products in this reaction exceeds the number of reactants; therefore, an effect of dilution would be expected.) In fact, when the product AMP is destroyed by addition of alkaline phosphatase to the reaction mixture (2), the reaction is still incomplete. This fact also argues that inhibition of transfer of amino acid by AMP is unlikely.

It is conceivable, of course, that a strong binding of charged tRNA by the Eₐ is responsible for the observed incomplete transfer of amino acid. In its simplest form, however, such a mechanism is unlikely, because in a typical tRNA charging reaction (i.e., starting with amino acid and enzyme, not with Eₐ) the enzyme does turn over many times.

A feasible and testable explanation for the above phenomenon is that aminoacyl-tRNA reacts with Eₐ to somehow block the amino acid transfer from Eₐ. However, the presence of free amino acid or ATP can somehow prevent or reverse this. Experiments are now in progress to check this possibility.

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