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# Species Specificity of Transfer RNA

ROBERT B. LOFTFIELD and ELIZABETH ANN EIGNER

*John Collins Warren Laboratory of the Collis P. Huntington Memorial Hospital of Harvard University, Massachusetts General Hospital, Boston, Massachusets, USA*

New techniques have permitted the study of the kinetics of the aminoacylation of transfer ribonucleic acid (S-RNA) by valine and isoleucine. It is observed that a single amino acid activating enzyme catalyzes the aminoacylation of S-RNA from different species at greatly different rates, but that the Michaelis constants are quite similar. This observation is confirmed by the demonstration of competition between different valine specific S-RNA's for the valine activating enzyme of *E. coli*.

We conclude that the enzyme recognition site and the code reading site of S-RNA may well be the same site and identical throughout all species. From these data there is no need to postulate separate sites or different codes. There is also no evidence of degeneracy of the code in these systems.

It is widely believed that the biosynthesis of protein proceeds through the following steps[1]:

$$AA_i + Ezy_i + ATP \rightleftharpoons Ezy_i\,(AA_i \sim AMP) + PP \quad (1)$$

$$Ezy_i\,(AA_i \sim AMP) + S\text{-}RNA_i \rightleftharpoons Ezy_i + AMP + AA_i \sim S\text{-}RNA_i \quad (2)$$

$$AA_i \sim S\text{-}RNA_i + AA_j \sim S\text{-}RNA_j \rightarrow AA_i\text{–}AA_j + S\text{-}RNA_i + S\text{-}RNA_j \text{ etc.} \quad (3)$$

where $AA_i$ is a particular natural L-amino acid, $Ezy_i$ is an enzyme specific for the activation of $AA_i$, ATP is adenosine triphosphate, $S\text{-}RNA_i$ is a molecule of transfer ribonucleic acid specific for $AA_i$, and $AA_i$–$AA_j$– represents the growing peptide chain. Reaction (3) is catalyzed by guanosine triphosphate[2] and microsomes together with enzymes which appear to show species specificity with regard to the microsomes but not with regard to the S-RNA[3,4]. On the other hand, Reaction (2) appears to show quite variable species specificity. Mammalian enzymes catalyze the synthesis of yeast valyl S-RNA[5] but not *E. coli* leucyl S-RNA[6]. Yeast or hog enzymes yield either yeast or hog tyrosyl S-RNA but not *E. coli* tyrosyl S-RNA while the *E. coli* enzyme cannot synthesize either yeast or hog tyrosyl S-RNA[7]. Yeast enzymes can react with only 40 % of the methionine

specific S-RNA of *E. coli*[8]. Leucine enzymes and leucine S-RNA's from various bacteria cross-react with each other but cannot cross-react with the leucine S-RNA's and leucine enzymes of yeast or mammals[4]. Finally, there is little or no cross-reaction between yeast and *E. coli* in the formation of tyrosyl or arginyl S-RNA, but there is cross-reaction in the formation of lysyl S-RNA[9].

The development of a very sensitive and accurate micro-assay for aminoacyl S-RNA in these laboratories[10] has permitted us to examine the kinetics of the synthesis of yeast and *E. coli* valyl S-RNA. We find that the heterologous enzymes and S-RNA's which superficially appear not to cross-react do, in fact, react almost as well as the homologous pairs as measured by Michaelis constants and inhibition constants.

## EXPERIMENTAL

L-$^{14}$C-valine and L-$^{14}$C-isoleucine were prepared from $Ba^{14}CO_3$ by way of the Bucherer hydantoin synthesis[11]. Each was at least 99.95 % pure. ATP and CTP were purchased from Sigma Chemical Company. S-RNA was isolated from baker's yeast or *E. coli* by the method of Monier *et al.*[5] Treatment at pH 10 for 30 minutes at 37°C (to remove bound amino acids) followed by dialysis increased the acceptor ability of the S-RNA by 2- to 10-fold. Mouse ascites "$S_4$ fraction" was prepared according to Hecht *et al.*[12] and *E. coli* activating enzymes were prepared by modifications of the method of Bergmann *et al.*[13] The enzymes were assayed by the hydroxamate method of Loftfield and Eigner[14], one unit of activity being the ability to form 1 $\mu\mu$mole of hydroxamate per hour under saturation conditions.

*Technique.* Typically 150 $\mu$l 0.1 M ATP-Mg, 150 $\mu$l 1.0 M tris buffer pH 7.6, 150 $\mu$l 0.84 mM $^{14}$C-valine ($11.2 \times 10^6$ cpm per $\mu$mole), 50 $\mu$l 2 % bovine albumin and 25 $\mu$l enzyme solution (900 units) are combined. One hundred $\mu$l of this mixture and appropriate amounts of water are brought to 26°C in a water bath and various amounts of S-RNA solution are added to give a total volume of 325 $\mu$l. Four aliquots of 75 $\mu$l are removed at intervals from 1 to 30 min and immediately pipetted into 5 ml of cold 5 % trichloroacetic acid (TCA). After 5 min the nearly clear suspension is filtered through a No. AAWG 02400 Millipore filter (Millipore Filter Corporation, Bedford, Mass., U. S. A.). The filter disk is washed twice with 5 % TCA and dried in a 60°C oven for 15 min before being assayed in a low background end window Geiger counter (Nuclear Chicago Corporation). Under these conditions S-RNA is quantitatively recovered over a range of 1 to 200 $\mu$g and there is no significant self absorption of $\beta$-rays if the weight of the combined RNA and protein is less than 1 mg[10].

## RESULTS

Fig. 1 shows that per unit of enzyme, *E. coli* valine enzyme acylates *E. coli* S-RNA about 500 times more rapidly than it does yeast S-RNA while $S_4$ enzyme is about 120 times more active toward yeast S-RNA than *E. coli* S-RNA. Curves E and F show that there is no measurable "fixation" of $^{14}$C-valine into the *E. coli* enzyme preparation and that the amount of such "fixation" into the $S_4$ preparation was only 3 % of that in Curve C. Curves C and D did not achieve the level of acylation shown by Curves A and B. If $S_4$ enzyme is added to the incubation of Curve C at 0 or 15 min the incorporation of $^{14}$C-valine quickly rises to the level of Curve B indicating that both enzymes acylate the same site of yeast S-RNA and that there has been no deterioration of the S-RNA. On the other hand, after 1 h of incubation with *E. coli* enzyme, only half of the yeast S-RNA is acylatable. This suggests that incubation with the *E. coli* enzyme results in some deterioration of yeast S-RNA which accounts for our failure to achieve maximum acylation of S-RNA by heterologous enzymes.

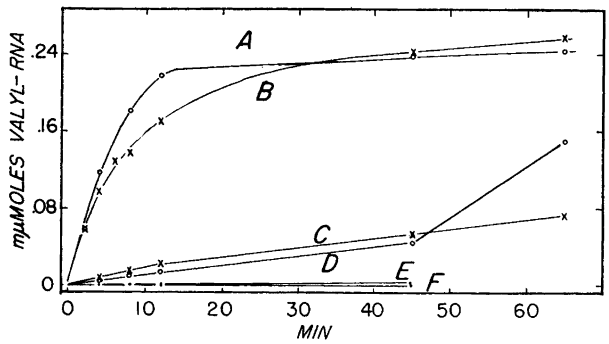


*Fig. 1.* Rate of formation of yeast or *E. coli* valine RNA by $S_4$ or *E. coli* activating enzymes. Curve A, 260 μg *E. coli* S-RNA and 190 units *E. coli* valine enzyme. Curve B, 300 μg yeast S-RNA and 610 units $S_4$ enzyme. Curve C, 300 μg yeast S-RNA and 3800 units of *E. coli* valine enzyme. Curve D, 260 μg *E. coli* S-RNA and 3050 units of $S_4$ enzyme. Curve E, 3050 units $S_4$ enzyme, no added RNA. Curve F, 3800 units *E. coli* valine enzyme, no added RNA. Total volume, 325 μl. At 45 min additional enzyme was added in each case. Other conditions as described under Experimental.

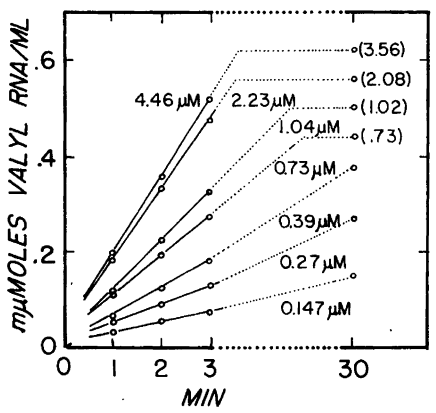


*Fig. 2.*

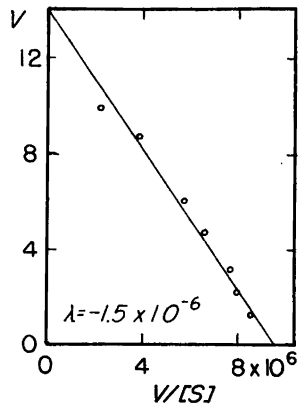


*Fig. 3.*

*Fig. 2.* Typical experiment showing RNA concentration dependence for aminoacylation of RNA. 570 units of *E. coli* valine enzyme were used, other conditions as described in the text except that the concnteration of *E. coli* S-RNA was varied from 0.103 mg/ml to 3.10 mg/ml. Based on the maximal incorporation of $^{14}C$-valine observed at 50 min, this corresponded to a molarity of valine specific S-RNA from 147 mμM to 4.46 μM.

*Fig. 3.* Eadie plot of rate data of Fig. 2. V is expressed in mμmoles valyl S-RNA formed per h per ml.

Combinations A, C and D were inhibited by between 20 % and 60 % by the presence of CTP, phosphoenol pyruvate and pyruvate kinase, so these substances were omitted from all the subsequent experiments.

By choosing appropriate concentrations of enzyme and S-RNA, it is possible to get quite good estimates of initial rates of reaction as shown in Fig. 2 for the

reaction of *E. coli* valine S-RNA with *E. coli* valine enzyme. From the specific activity of the $^{14}$C-valine (11.2 cpm per $\mu\mu$mole) and the maximal incorporation of valine into this S-RNA, one calculates that this S-RNA reacts with 1.41 m$\mu$-moles of valine per mg. In turn, this permits the estimation of the concentration of valine specific S-RNA. Plotting the velocity of the reaction against the velocity divided by substrate concentration[15], we find in Fig. 3 a typical Michaelis-Menten relationship with a $K_m$ of $1.5 \times 10^{-6}$. Comparable experiments indicate that *E. coli* valine S-RNA and yeast valine S-RNA react with $S_4$ enzyme with $K_m$'s of about $1.3 \times 10^{-6}$ and $2.5 \times 10^{-7}$ respectively. Yeast valine S-RNA reacts very slowly with *E. coli* valine enzyme but a rough approximation to the $K_m$ is $2.5 \times 10^{-6}$. The $K_m$ for *E. coli* isoleucine S-RNA on *E. coli* isoleucine enzyme is $1.2 \times 10^{-6}$.

In every case we noted that the rate of aminoacylation decreases more rapidly than would be predicted from the progressive decrease in the amount of substrate. This is probably due to inhibition of the enzyme by the product. The rate of $^{14}$C-valine addition to *E. coli* S-RNA is reduced by 20 % in the presence of a 6-fold greater concentration of $^{12}$C-valine saturated S-RNA. A 10-fold excess of $^{12}$C-isoleucine saturated S-RNA suppressed the reaction of $^{14}$C-isoleucine by 75 %. Since similar effects were not observed for unacylated S-RNA, we consider these to be due to product inhibition.

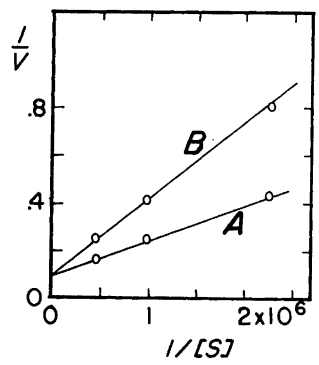


*Fig. 4.*

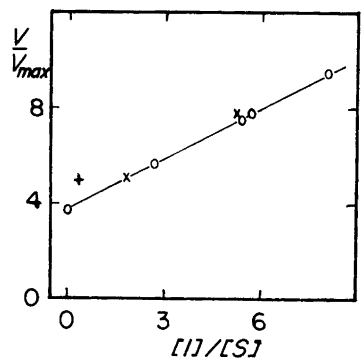


*Fig. 5.*

*Fig. 4.* Competition between yeast S-RNA and *E. coli* S-RNA for *E. coli* valine activating enzyme. Each inhibited reaction contained 600 $\mu$g of yeast S-RNA (2.78 $\mu$M of valine specific S-RNA) and all reactions included 238 units of *E. coli* valine enzyme in 325 $\mu$l of solution. Other conditions and components as described in the experimental section except that the *E. coli* S-RNA concentration was varied from 315 $\mu$g/ml to 1.57 mg/ml (0.44 $\mu$M to 2.23 $\mu$M of valine specific S-RNA). Curve A uninhibited. Curve B inhibited. $K_m = 1.5 \times 10^{-6}$, $K_i = = 2.1 \times 10^{-6}$. Units of $V$ are m$\mu$moles of valyl S-RNA formed per h per ml.

*Fig. 5.* Dependence of yeast S-RNA inhibiting ability on concentration of valine specific yeast S-RNA. Each tube contained 361 $\mu$g *E. coli* S-RNA (0.51 $\mu$M of valine specific S-RNA), 190 units of *E. coli* valine enzyme, other components as described in the text and the following yeast S-RNA's: O, 0 to 2.76 mg/ml of ordinary yeast S-RNA (0 to 4.2 $\mu$M valine acceptor S-RNA); ×, 152 and 412 $\mu$g/ml of enriched valine acceptor yeast S-RNA[16] (0.84 or 2.68 $\mu$M valine acceptor sites); or +, 3.2 mg/ml of enriched tyrosine and serine acceptor yeast S-RNA containing less than 1 % of the usual valine acceptor ability (0.08 $\mu$M valine acceptor sites).

If yeast S-RNA is capable of being aminoacylated by *E. coli* valine enzyme at a very slow rate, it would be expected that yeast S-RNA would be inhibitory to the aminoacylation of *E. coli* S-RNA. This effect is demonstrated in Fig. 4. In this plot the slope is equal to $(1 + [I]/K_i)K_s$. If the inhibitor, I, is considered to be only that part of the yeast S-RNA that will accept valine from $S_4$ enzyme, we may calculate $K_i$ to be $2.1 \times 10^{-6}$ in excellent agreement with the previously observed $K_s$ ($2.5 \times 10^{-6}$) for reaction of yeast valine S-RNA with *E. coli* valine enzyme.

If the usual equation for competitive inhibition is rewritten as $V_{max}/v = K_s/[S] + 1 + K_s [I]/K_i [S]$ a plot of $V_{max}/v$ *vs.* $[I]/[S]$ at constant substrate concentration should give a straight line of slope $K_s/K_i$. Fig. 5 shows that several concentrations of crude yeast S-RNA conform to this equation. The evidence of Fig. 4 and 5 is consistent with valine specific yeast S-RNA being a specific competitive inhibitor of valine specific *E. coli* S-RNA. It does not exclude the possibility that several or all components of yeast S-RNA are inhibitory. In order to test this possibility, yeast S-RNA enriched 5-fold in valine acceptor capability was prepared[16] and tested for inhibitor activity. It can be seen in Fig. 5 that the inhibitory activity was exactly proportional to the extent of enrichment in valine acceptor capability. It should however be noted that a massive concentration (3 mg/ml) of yeast S-RNA enriched in tyrosine and serine acceptor capability and depleted in valine activity[17] showed more inhibition than would have been predicted. At this high concentration of S-RNA there may be non-specific effects. Had the inhibition been due equally to all S-RNA molecules it should have been about 70 % at this concentration instead of only 25 %.

## DISCUSSION

If previously available methods had been used, we would surely have concluded that *E. coli* valine enzyme was inactive toward yeast S-RNA. From this, according to the absence of species specificity in Reaction (3), we might have concluded that the "code reading" area of the S-RNA is universal while the "activating enzyme recognition" site varies from species to species, hence that the two functions of S-RNA are distinct.

The present data argue to the contrary. From the relative Michaelis constants, the inhibition constants, and the data of Fig. 5, it is clear that valine specific yeast S-RNA associates with *E. coli* valine enzyme very nearly as well as *E. coli* S-RNA does. The great difference between these two S-RNA's is in the rate of reaction of the enzyme-substrate complex to yield valyl S-RNA. This difference in reaction rates is unquestionably a consequence of structural differences, but these are not necessarily or even likely in the enzyme recognition area. It is therefore not necessary to postulate that the "code reading" and "enzyme recognition" sites are distinct in S-RNA.

It should be observed that there is no evidence for degeneracy of the S-RNA in these experiments. Our techniques would have disclosed the presence of two different yeast or *E. coli* valine S-RNA's if they were present in similar amounts and had $K_m$'s differing by only a factor of two.

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Många, många goda år tillönskas Professor och fru Theorell av Robert Loftfield, som hade livets bästa år hos Professor Theorell året 1952–1953.

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