Studies Concerning the Interaction of Serine Soluble Ribonucleic Acid with Seryl Soluble Ribonucleic Acid Synthetase from Baker's Yeast*

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ABSTRACT: Studies of enzyme stability and of the kinetics of seryl soluble ribonucleic acid (seryl-s-RNA) formation have been carried out with use of purified yeast seryl-s-RNA synthetase and partially purified serine s-RNA preparations. The thermal stability of the enzyme was not affected by adenosine triphosphate (ATP) alone or by serine-specific yeast s-RNA in the presence or absence of Mg^{2+}; however, Mg^{2+} appreciably increased thermal stability and ATP plus Mg^{2+} was more effective than Mg^{2+} alone.

The kinetics of seryl-s-RNA formation catalyzed by the purified enzyme were studied with both yeast and *Escherichia coli* s-RNA substrates: with the *E. coli* substrate, K_m for s-RNA was higher, maximum velocity lower, and the magnitude of inhibition produced by phosphate ions greater than obtained with yeast s-RNA substrate. The kinetics of the reaction with adenosine-terminal serine-specific yeast s-RNA as substrate were not significantly affected by the presence of cytidine-terminal (*i.e.*, lacking the terminal adenosine) serine-specific s-RNA, suggesting a major role of the terminal adenosine in the interaction of serine s-RNA with the enzyme complex. Removal of phosphate from the guanosine terminal of yeast serine s-RNA did not alter reaction rates with this substrate. Under optimal conditions *E. coli* and yeast enzyme fractions catalyzed the same total formation of seryl-s-RNA with crude *E. coli* s-RNA as substrate, but maximal formation with purified yeast enzyme required the additional presence of an enhancing factor. The enhancing factor, partially purified from yeast, has been characterized in preliminary fashion.

The preparation and some properties of crystalline seryl soluble ribonucleic acid (seryl-s-RNA) synthetase from yeast have been described in an earlier paper (Makman and Cantoni, 1965). This paper will present studies on the influence of s-RNA and other substrates upon the stability of the enzyme and on the kinetics of seryl-s-RNA formation under various conditions and with various s-RNA species as substrates. The kinetic studies, carried out with purified preparations both of s-RNA and of enzyme, have yielded quantitative information concerning some of the structural parameters of importance in the interaction between the enzyme and its s-RNA substrate.

Experimental Section

Preparation and Assay of Enzymes. Crystalline yeast seryl-s-RNA synthetase (step 5, 55% saturated ammonium sulfate fraction) was prepared and assayed as described previously (Makman and Cantoni, 1965). An extract containing crude seryl-s-RNA synthetase was prepared from *Escherichia coli* by high-speed centrifugation as described by Bennett *et al.* (1963).

s-RNA Preparations. Preparations of s-RNA from yeast (Apgar *et al.*, 1962) and rat liver (Cantoni *et al.*, 1962) were carried out in collaboration with H. H. Richards. Serine-specific s-RNA from yeast was prepared by repeated chromatography on Sephadex (Tanaka *et al.*, 1962). For some of the studies reported below, we used a preparation of yeast serine s-RNA which had been fractionated further by countercurrent distribution with 300 transfers in the solvent system of Doctor and Connelly (1961). Countercurrent distribution resulted in the resolution of yeast serine s-RNA into two species which differ by the presence and absence of the terminal adenosine residue.[1]

Average *E. coli* s-RNA and *E. coli* s-RNA enriched for serine-acceptor activity by application of partition chromatography were prepared and generously furnished by S. G. Nathenson (Nathenson *et al.*, 1965).

Resolution of *E. coli* serine s-RNA into two species, *E. coli* s-RNA-SerI and *E. coli* s-RNA-SerII, has been obtained with use of countercurrent distribution by Goldstein *et al.* (1964). We are greatly indebted to Drs. J. Goldstein, T. P. Bennett, and F. Lipmann for the generous gift of these highly purified preparations.

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[1] H. H. Richards, M. H. Makman, P. Lebowitz, P. L. Ipata, and G. L. Cantoni, to be published.

Measurement of Total Serine-Acceptor Capacity of s-RNA. The total serine-acceptor capacity of yeast and liver s-RNA was measured as described previously (Makman and Cantoni, 1965). In order to attain maximal formation of seryl-s-RNA with *E. coli* s-RNA as substrate for yeast seryl-s-RNA synthetase, the incubation was carried out at pH 8.1 (measured with glass electrode) with 0.02 M glycine as buffer.

Assay of Enhancing Factor. The conditions described above for measurement of total serine-acceptor capacity of *E. coli* s-RNA were also used for measurement of the "enhancing factor" described in this paper. Average *E. coli* s-RNA (18 absorbancy units/ml) and a saturating amount of yeast seryl-s-RNA synthetase were present. Activity represents the additional seryl-s-RNA formation due to presence of enhancing factor (*i.e.*, seryl-s-RNA formation in the presence of enhancing factor plus excess synthetase minus seryl-s-RNA formation in the presence of excess synthetase only). Assay of crude extracts was possible since extracts could be diluted so that the amount of seryl-s-RNA synthetase in the extracts was negligible, and yet show enhancing factor activity.

Measurement of Rate of Aminoacyl-s RNA Formation. Kinetic studies were carried out with either Tris or potassium phosphate buffer (0.05 M, pH 7.5), 0.065 μmole/ml of [^{14}C]serine (specific activity, 20 μc/μmole), 0.005 M ATP[2] (sodium salt, neutralized), 0.013 M $MgCl_2$, 0.004 M reduced glutathione, 0.2 mg/ml of bovine serum albumin (twice recrystallized), and a series of different concentrations of s-RNA. Incubation times varied from 1.0 to 5 min: for each set of s-RNA and enzyme concentrations, two measurements at different incubation times were made in order to be certain that initial rates were measured. The values for serine s-RNA substrate concentration represent the total serine-acceptor capacity of the s-RNA preparation as determined separately under identical conditions of incubation, but with excess time of incubation and a saturating level of seryl-s-RNA synthetase.

Other conditions for assay and measurement of [^{14}C]aminoacyl-s-RNA formation with use of liquid scintillation spectrometry were as described earlier (Makman and Cantoni, 1965).

Results

Heat Inactivation Studies. Yeast seryl-s-RNA synthetase is very labile to heat. At 45° there is rapid inactivation in the presence or absence of EDTA (EDTA has a stabilizing effect at 37° or lower temperatures) (Makman and Cantoni, 1965). Table I lists the results of experiments in which various components of the enzyme assay system were tested individually or in combination for their ability to reduce the rate of heat inactivation at 47°. Addition of ATP alone had no effect on heat inactivation. However, a marked increase in heat stability occurred in the presence of magnesium ions. Magnesium plus ATP was more effective than magnesium alone. Concentrations of Mg^{2+} and ATP as low as 0.0026 and 0.0013 M, respectively, exerted protective effect. Since the purified synthetase itself appears to contain bound nucleotide (Makman and Cantoni, 1965), the observation that Mg^{2+} is required for the protective effect of ATP suggests that the effect obtained with Mg^{2+} alone may really be due to Mg^{2+} plus bound nucleotide.

TABLE I: Stability of Seryl-s-RNA Synthetase to Incubation at 47°.

Incubn for 10 min with[a]	% Init Act.[b]
Control	20
s-RNA_{Ser}	20
$MgCl_2$	60
$MgCl_2$ + s-RNA_{Ser}	62
ATP	24
ATP + $MgCl_2$	96
ATP + $MgCl_2$ + s-RNA_{Ser}	75
ATP + $MgCl_2$ + serine	100
ATP + $MgCl_2$ + serine + s-RNA_{Ser}	68

[a] Crystalline seryl-s-RNA synthetase (3 mg/ml, suspended in 55% saturated ammonium sulfate) was diluted 1:150 in a solution containing 0.05 M potassium phosphate buffer (pH 7.5), 2.5 mg/ml of gelatin, and 0.006 M EDTA. Aliquots were incubated at 47° for 10 min prior to enzyme assay. Other additions were present where indicated during the 47° incubation at the following concentrations: s-RNA_{Ser} (serine-specific s-RNA from yeast, purified by partition chromatography on Sephadex), 8 absorbancy units (260 mμ)/ml; $MgCl_2$, 0.024 M; ATP, 0.005 M; serine, 16 μM. [b] Correction has been made for the effect of addition of serine and s-RNA_{Ser} on the subsequent enzyme assay.

Yeast serine s-RNA was found to have no effect on heat stability, either when present alone or in combination with magnesium (Table I). Such a result might have been anticipated since according to current formulation of the reaction mechanism serine s-RNA reacts with the seryl adenylate–enzyme complex rather than with the enzyme itself. Actually, in the presence of ATP plus Mg^{2+}, serine s-RNA resulted in a decrease in the heat stability of the enzyme. The specificity of this effect has not been established. It is also not known whether it represents an interaction of the s-RNA with the enzyme in the presence of ATP plus Mg^{2+} or merely interference with the binding of ATP plus Mg^{2+} to the enzyme.

Kinetic Studies

Seryl-s-RNA Formation with s-RNA from Yeast and from E. coli. The kinetics of seryl-s-RNA formation catalyzed by purified yeast synthetase were studied with

[2] Abbreviations used: ATP, adenosine triphosphate; AMP, adenosine monophosphate.

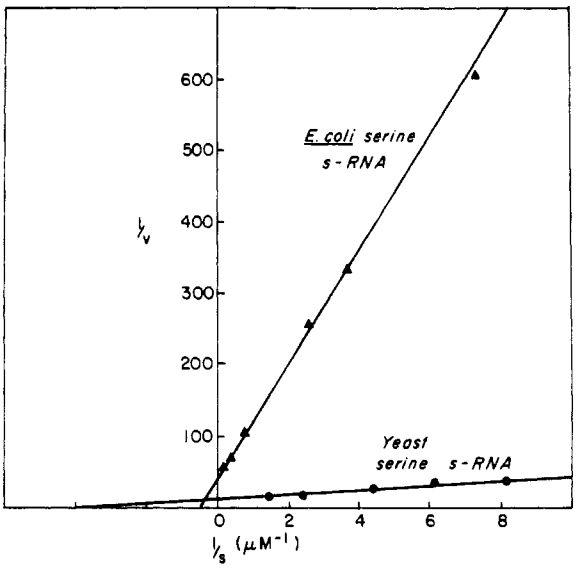

FIGURE 1: Lineweaver–Burk plot for yeast seryl-s-RNA synthetase: comparison of yeast and *E. coli* s-RNA substrates. Velocity (v) is expressed as micromoles of seryl-s-RNA formed per minute per liter. Concentration of serine s-RNA (S) is expressed as micromoles per liter of s-RNA capable of accepting serine (with excess purified yeast enzyme present). Total serine acceptor capacities of the yeast s-RNA and *E. coli* s-RNA used in this experiment were 0.355 mμmole/absorbancy unit (260 mμ) and 0.125 mμmole/absorbancy unit (260 mμ), respectively. The serine s-RNA preparations were prepared with use of Sephadex partition chromatography and the assays carried out as described under Experimental Section. The buffer used for the incubations was Tris, 0.05 M, pH 7.5.

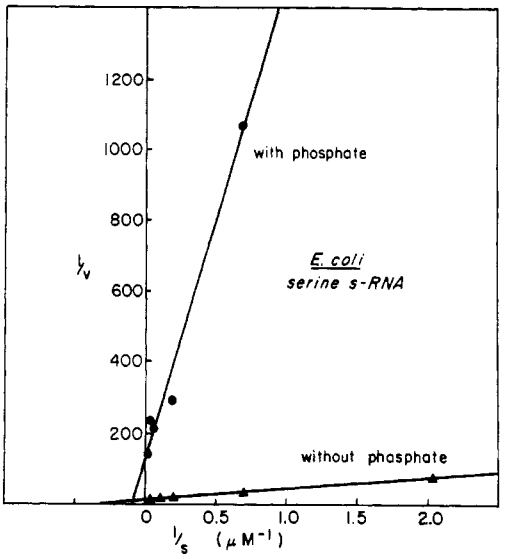

FIGURE 2: Lineweaver–Burk plot for yeast seryl-s-RNA synthetase: effect of phosphate on reaction rates with *E. coli* s-RNA as substrate. Velocity (v) is expressed as micromoles of seryl-s-RNA formed per minute per liter. Concentration of *E. coli* serine s-RNA (prepared by Sephadex partition chromatography) (S) is expressed as micromoles per liter of s-RNA capable of accepting serine (with excess enzyme present and in the absence of phosphate). The assay incubations were carried out as described under "Experimental Section" in the presence of Tris buffer (0.05 M, pH 7.5) either without phosphate present (▲—▲) or with 0.02 M potassium phosphate (pH 7.5) present in addition to the Tris buffer (●—●).

both yeast and *E. coli* s-RNA substrates. Figure 1 shows a plot of the reciprocal of velocity *vs.* the reciprocal of substrate concentration (Lineweaver and Burk, 1934) for the two substrates. For this experiment, incubations were carried out in Tris buffer and the s-RNA substrates used were fractions obtained by Sephadex partition chromatography: the yeast s-RNA had been rechromatographed on Sephadex columns and accepted only serine; the *E. coli* s-RNA was the peak serine fraction obtained by Sephadex partition chromatography and contained leucine s-RNA in addition to serine s-RNA. The *E. coli* s-RNA was found to have a lower affinity for the enzyme (K_m was about 10-fold greater than that for yeast s-RNA). Also, the maximum velocity obtained with the *E. coli* s-RNA was only about one-fourth that obtained with yeast s-RNA as substrate. These values are summarized in Table II.

Since the incubation mixture initially used for assay of yeast seryl-s-RNA synthetase contained phosphate buffer (Makman and Cantoni, 1965), investigation was made of the possible influence of phosphate ions on the kinetics of seryl s-RNA formation. These studies revealed another difference between yeast and *E. coli* serine s-RNA substrates. Although phosphate ions produced inhibitory effects on seryl-s-RNA formation with the two substrates that were qualitatively similar, the magnitude of the effects with *E. coli* substrate were very much greater (Table III). The presence of phosphate resulted in both an increased K_m and a decreased maximum velocity for the *E. coli* substrate, as is shown in Figure 2. Most striking is the change in maximum velocity, which in the presence of phosphate is only one-tenth that obtained without phosphate.

Requirement for the Terminal Adenosine of s-RNA in the Interaction of s-RNA with Enzyme. A high proportion of yeast s-RNA is often found to be lacking the terminal adenosine (*e.g.*, Table IV in Makman and Cantoni, 1965) (Furth *et al.*, 1961). It was of interest to determine whether or not s-RNA chains which lacked the terminal adenosine, although unable to accept amino acid, could exert an effect on the kinetics of formation of aminoacyl-s-RNA. In recent studies in this laboratory[1] it has been possible to resolve purified yeast serine s-RNA into two components: one terminating in CCA, and the other terminating in CC (lacking the terminal adenosine) (Figure 3). Moreover, CC-terminal serine s-RNA could be converted to a CCA-terminal

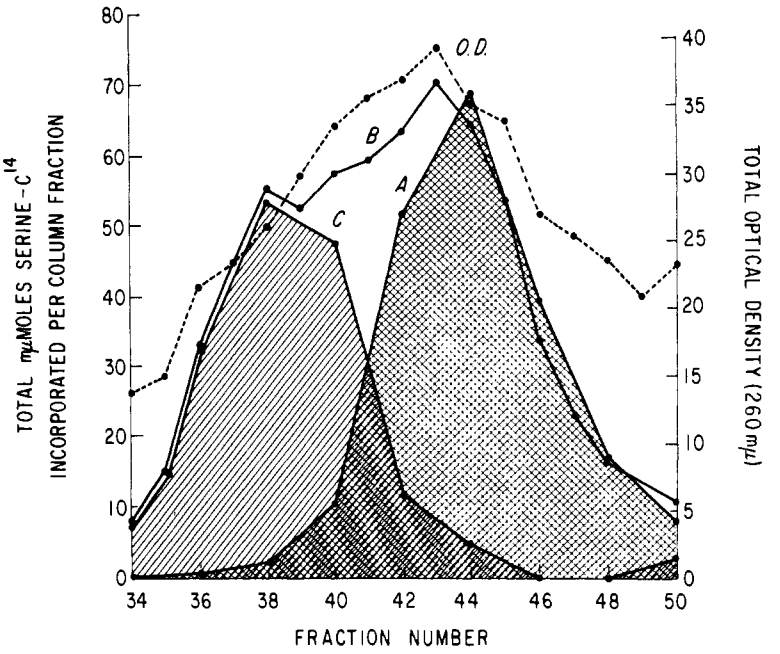

FIGURE 3: Separation of cytidine- and adenosine-terminal species of serine-specific s-RNA of yeast by countercurrent distribution (see text for conditions). The early portion of the serine s-RNA region from a Sephadex chromatogram was used for this experiment. Curve A (cross-hatched area): assay of fractions with purified seryl-s-RNA synthetase alone. Curve B: assay with seryl-s-RNA synthetase plus CCA-pyrophosphorylase. Curve C (diagonally shaded area): the difference between areas under B and A, and indicating the amount of C-terminal s-RNA present.

TABLE II: K_m Values and Turnover Numbers for Yeast and *E. coli* s-RNA.[a]

Substrate	K_m (μM)[b]	Turnover No. (Moles of Seryl-s-RNA/min per Mole of Enzyme)[c]
Yeast serine s-RNA	0.24	50
E. coli serine s-RNA	2.6	12

[a] For measurement of rates of seryl-s-RNA formation, incubations were carried out with Tris buffer, yeast, or *E. coli* serine-specific s-RNA (purified with use of Sephadex partition chromatography) and other assay components as indicated in the text. [b] Concentrations of s-RNA represents only that which is capable of accepting serine in the presence of excess enzyme. [Total serine-acceptor capacities (average values) of the yeast s-RNA and *E. coli* s-RNA used in these experiments were 0.42 mμmole/absorbancy units (260 mμ) and 0.15 mμmole/absorbancy unit (260 mμ), respectively.] [c] Purified yeast seryl-s-RNA synthetase, mol wt 89,000 (Makman and Cantoni, 1965).

species by incubation of the isolated CC-terminal s-RNA in the presence of ^{14}C-labeled ATP and partially purified CCA-pyrophosphorylase, followed by reisolation by countercurrent distribution; the partition coefficient of the labeled s-RNA product was now identical with that of the native CCA-terminal species.[1] The separated CC and CCA species of serine s-RNA have been used for investigation of the importance of the terminal adenosine in the interaction of serine s-RNA with the seryl adenylate–enzyme complex.

A Lineweaver–Burk plot for CCA-terminal serine s-RNA is shown in Figure 4; the points for this plot are indicated by the circles. The triangles represent rates in the presence of 0.182 μM CC-terminal serine s-RNA.[3] Both species of s-RNA were essentially pure with regard to their amino acid acceptor capacity. The presence of the CC species caused no detectable change in the kinetics of the reaction. If the CC-terminal s-RNA had the same affinity for the enzyme as the CCA-terminal species (*i.e.*, if $K_i = K_m$) and thereby acted as a competitive inhibitor, in the presence of the CC species the slope K_m/V_{max} would be increased as shown by the broken line. This experiment indicates that the affinity

[3] The concentration of the CC-terminal species was determined as the total serine-acceptor capacity obtained when both the purified synthetase and purified CCA-pyrophosphorylase were present.

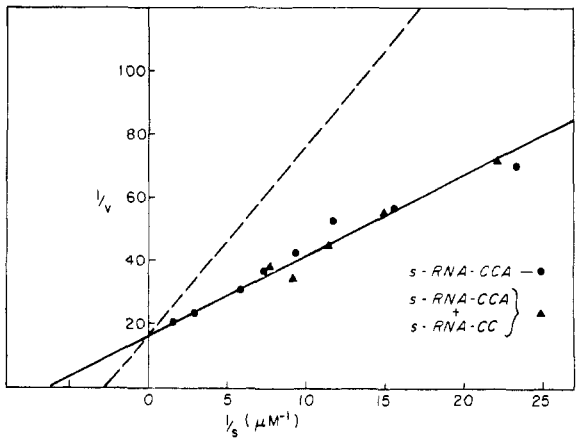

FIGURE 4: Lineweaver–Burk plot for yeast seryl-s-RNA synthetase: lack of effect of CC-terminal serine s-RNA. Velocity (v) is expressed as micromoles of seryl-s-RNA formed per minute per liter. Concentration of serine s-RNA (s) is expressed as micromoles per liter of s-RNA capable of accepting serine (the adenosine-terminal s-RNA accepted approximately 1 mole of serine/mole of s-RNA; see Figure 3). The yeast serine-specific s-RNA which contained the terminal adenosine (s-RNA-CCA) and that which lacked the terminal adenosine (s-RNA-CC) were prepared and the assay incubations (in Tris buffer) carried out as described under Experimental Section. The circles (●) represent rates for the s-RNA-CCA substrate alone. The triangles (▲) represent rates for different amounts of s-RNA-CCA all in the presence of 0.182 μM s-RNA-CC (acceptor capacity dependent upon the presence of CCA-pyrophosphorylase; see Figure 3). If s-RNA were to have the same affinity for the enzyme as s-RNA-CCA and thereby to act as a competitive inhibitor (with $K_i = K_m$), in the presence of s-RNA-CC, the slope (K_m/V_{max}) would be increased as shown by the dashed line (-----).

of the CC species is less than one-tenth that of the CCA-terminal s-RNA species.

Consistent with the above findings is the similarity of the K_m for serine s-RNA measured with the purified CCA-terminal serine s-RNA ($K_m = 0.16 \times 10^{-6}$ M; Figure 4) to the K_m obtained with s-RNA fractionated by partition chromatography on Sephadex only and containing 30–90% of serine s-RNA with CC-terminal chains (average $K_m = 0.26 \times 10^{-6}$ M; Table II).

Lack of Effect of Removal of Phosphate from the Guanosine Terminal of s-RNA. It has previously been shown by Harkness and Hilmoe (1962) and by Fiers and Khorana (1963) that removal of the terminal phosphate from the pGp end of average yeast s-RNA does not decrease the amino acid acceptor capacity of the s-RNA. Although these studies indicated that the terminal 5′-monoester phosphate group of s-RNA was not necessary for acceptor function, the possibility still remained that this terminal phosphate might influence the reaction rate. In order to investigate this possibility for the reaction catalyzed by purified seryl-s-RNA synthetase, we have examined the kinetics of seryl-s-RNA formation with yeast serine s-RNA treated with *E. coli* phosphomonoesterase (Torriani, 1960; Garen and Levinthal, 1960) in order to remove the guanosine-terminal phosphate. The results obtained indicated that the removal of this phosphate did not alter the kinetics of the reaction: the values for K_m and maximum velocity obtained with the phosphomonoesterase-treated s-RNA substrate differed by <10% from those obtained with native serine s-RNA as substrate. Thus, the 5′-terminal phosphate does not appear to play a role in the interaction of serine s-RNA with the seryl adenylate–enzyme complex.

TABLE III: Effect of Phosphate on Rate of Formation of Seryl-s-RNA.

Serine s-RNA (μM)	μmoles of Seryl-s-RNA[a]/l. min: Without PO_4	With 0.02 M PO_4	% Inhib
Yeast s-RNA			
1.22	0.135	0.105	22
4.05	0.215	0.171	20
12.15	0.194	0.188	3
E. coli s-RNA			
1.47	0.007	0.001	86
14.7	0.069	0.005	93
29.4	0.086	0.007	92

[a] For measurement of rates of seryl s-RNA formation incubations were carried out with Tris buffer (pH 7.5) both without and with 0.02 M potassium phosphate (pH 7.5) also present, yeast or *E. coli* serine-specific s-RNA, and other assay components as indicated in the text.

Total Acceptor Capacity of s-RNA for Serine. SPECIES SPECIFICITY. It has been shown that the interaction between an aminoacyl-s-RNA synthetase and the corresponding amino acid specific s-RNA can be species specific, although this is not always the case (Berg *et al.*, 1962; Benzer and Weisblum, 1961; Rendi and Ochoa, 1962; Bennett *et al.*, 1963; Doctor and Mudd, 1963). In the presence of purified seryl-s-RNA synthetase plus purified yeast CCA-pyrophosphorylase[4] s-RNA preparations (total, unfractionated s-RNA) from yeast and rat liver had similar total acceptor capacities for serine. The rat liver s-RNA had about the same serine-acceptor capacity whether the purified yeast enzymes or a liver pH 5 enzyme fraction (Cantoni *et al.*, 1962) was used.

The conditions for maximal formation of seryl-s-RNA

[4] P. Lebowitz, P. L. Ipata, M. H. Makman, and G. L. Cantoni, to be published.

with *E. coli* s-RNA as substrate were more stringent than with yeast s-RNA as substrate. With the yeast substrate, it was possible to achieve maximal seryl-s-RNA formation with a [^{14}C]serine concentration of 0.02 μmole/ml while with the *E. coli* substrate it was necessary to use a serine concentration of about 0.06 μmole/ml or greater for maximal seryl-s-RNA formation. The maximal incorporation of [^{14}C]serine into the *E. coli* substrate was appreciably greater (about twofold) when the reaction was carried out at pH 7.9–8.1 with glycine buffer instead of either the standard phosphate or Tris buffer at pH 7.5. The need for higher enzyme concentrations and/or longer incubation times with the *E. coli* substrate (generally 60 min of incubation with 1 μg/ml of purified seryl-s-RNA synthetase was adequate) was not unexpected in view of the differences found in kinetic studies with the yeast and *E. coli* s-RNA substrates.

The total serine-acceptor capacity of s-RNA from *E. coli* measured with enzymes from *E. coli* and yeast is shown in Table IV. Under the optimal conditions discussed above, only about 40% of the *E. coli* serine s-RNA chains available to crude extracts of *E. coli* could be acylated by crystalline yeast seryl-s-RNA synthetase. However, addition of another yeast fraction (enhancing factor), itself devoid of seryl-s-RNA synthetase activity, enabled the crystalline yeast synthetase to charge the *E. coli* serine s-RNA as completely as the *E. coli* enzyme (Table IV). Enhancing activity could be detected in crude extracts from both *E. coli* and yeast, and was obtained in purified form in the ammonium sulfate fraction containing arginyl-s-RNA synthetase (step 5 fraction of Makman and Cantoni, 1965).

TABLE IV: Maximal Serine Incorporation into *E. coli* s-RNA.[a]

Enzyme Addn	μmoles of [^{14}C]Serine Incorpd/OD_{260} Unit of s-RNA[b] × 10^6
E. coli enzyme[c]	45.6
Yeast seryl-s-RNA synthetase	18.3
Yeast seryl-s-RNA synthetase + yeast enhancing factor[d]	48.8
Yeast enhancing factor[d]	0
Yeast seryl-s-RNA synthetase + *E. coli* enzyme[c]	45.6
E. coli enzyme[c] + yeast enhancing factor	46.7

[a] Unfractionated *E. coli* s-RNA, 18 absorbancy units/ml. Assay was carried out as described in the text. [b] Absorbancy unit at 260 mμ. [c] The supernatant fraction of *E. coli* prepared following sonic disintegration of cells by high-speed centrifugation as described by Bennett *et al.* (1963). [d] The step 5 arginyl-s-RNA synthetase fraction (55% saturated ammonium sulfate extract) from yeast (Makman and Cantoni, 1965).

The enhancing factor has been characterized in only a preliminary fashion. It is heat labile and not dialyzable. Activity (enhancement of serine acceptor capacity) was not demonstrable when assays were carried out with yeast s-RNA in place of *E. coli* s-RNA. The yeast arginyl-s-RNA synthetase fraction which contains the factor in purified form did not contain detectable amounts of CCA-pyrophosphorylase activity assayed either by incorporation of AMP from [^{14}C]ATP into yeast and *E. coli* s-RNA, or by restoration of serine acceptor capacity of cytidine-terminal yeast s-RNA under various assay conditions. The nonidentity of the enhancing factor with CCA-pyrophosphorylase is evidenced by two additional observations: (1) a preparation of partially purified CCA-pyrophosphorylase activity from yeast was inactive in the assay for enhancing factor; and (2) sucrose-gradient centrifugation (Figure 5) and Sephadex G-100 and G-200 column fractionation of yeast extracts have revealed that two separate protein fractions are responsible for these two activities.

Although it appeared possible to achieve the same total incorporation of serine into *E. coli* s-RNA with yeast enzyme fractions as with an *E. coli* enzyme fraction, the question remained as to whether or not the serine was incorporated into the same s-RNA molecules in both cases. The addition of yeast seryl-s-RNA synthetase (with or without enhancing factor) to the incubation system already containing *E. coli* enzyme caused no further incorporation of serine into *E. coli* s-RNA above that obtained with *E. coli* enzyme alone. Also, in an experiment carried out in collaboration with S. G. Nathenson, crude *E. coli* s-RNA was fractionated by partition chromatography on Sephadex (Tanaka *et al.*, 1962), and the elution pattern for serine s-RNA was determined by assay of each fraction with crude *E. coli* enzyme, and with purified yeast seryl-s-RNA synthetase both with and without the enhancing factor from yeast; the serine s-RNA peaks obtained by these three assay procedures were superimposable. These experiments indicated that both yeast and *E. coli* enzymes catalyzed the addition of serine to the same *E. coli* s-RNA molecules.

The above results differ from the findings of Bennett *et al.* (1963). These investigators have reported that crude yeast enzymes are able to catalyze seryl-s-RNA formation with only about 60% of the *E. coli* s-RNA chains which are available to seryl-s-RNA synthetase derived from *E. coli*. More recently these investigators have reported studies of two *E. coli* serine s-RNA species; these species (s-RNA-SerI and s-RNA-SerII) were separated by countercurrent distribution and shown to have different coding characteristics in a polynucleotide-stimulated, protein-synthesizing system. Yeast enzyme was found to charge s-RNA-SerI with serine as well as did *E. coli* enzyme, but s-RNA-SerII

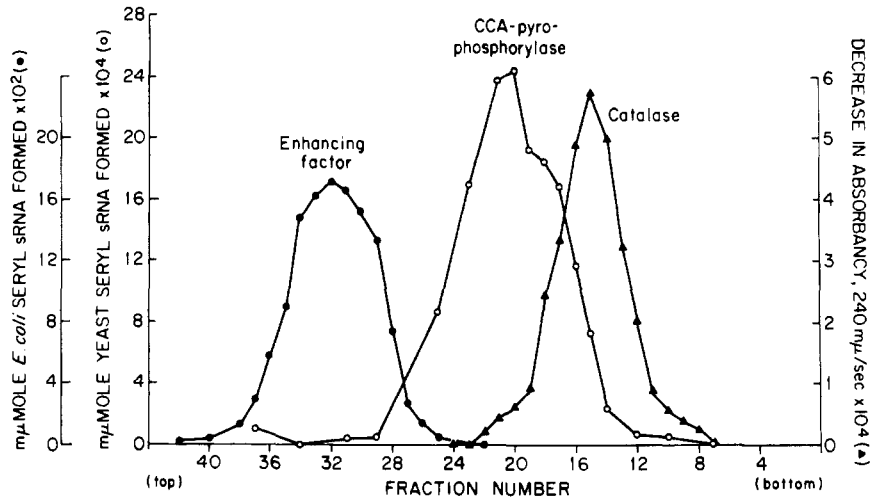

FIGURE 5: Resolution of enhancing factor and CCA-pyrophosphorylase activity of yeast by sucrose-density gradient centrifugation. Density-gradient centrifugation (Martin and Ames, 1961) of a 45–55% saturation ammonium sulfate fraction of yeast (1.2 mg of protein) was carried out at 38,000 rpm for 13.6 hr at 3° in a Spinco SW 39 rotor with a linear gradient of 5–20% sucrose and with 0.06 M phosphate buffer (pH 7.4). Fractions of 7 drops each were collected from the bottom of the tube for assay. Beef liver catalase was used as a marker (▲—▲). For assay of CCA-pyrophosphorylase, conditions were as previously described for measurement of seryl-s-RNA synthetase (Makman and Cantoni, 1965) with the following exceptions: average yeast s-RNA containing a high proportion of cytidine-terminal chains (acceptor activity for serine was stimulated eight fold in the presence of maximal CCA-pyrophosphorylase) was used (25 absorbancy units/ml) and a saturating amount of yeast seryl-s-RNA synthetase was present. Activity represents seryl-s-RNA formation due to the presence of CCA-pyrophosphorylase (●—●). The assay of enhancing factor was carried out as described in the text. Activity represents seryl-s-RNA formation due to the presence of enhancing factor (●—●) (0.11 mμmole of seryl-s-RNA, formed in the absence of enhancing factor, has been subtracted from each value). All activities are per microliter of gradient fraction.

was only partially charged (Goldstein *et al.*, 1964).

A further examination of the formation of seryl-s-RNA with *E. coli* s-RNA and yeast enzyme was made possible by the generosity of Drs. Bennett, Goldstein, and Lipmann who made available to us samples of the two serine s-RNA fractions from *E. coli*. In the presence of purified synthetase plus enhancing factor from yeast, the extent of formation of seryl-s-RNA was 79% of that which occurred with crude *E. coli* enzyme for s-RNA-SerI, and 89% for s-RNA-SerII (Table V), *i.e.*, approximately the same for the two s-RNA species. With purified yeast synthetase alone, the extent of seryl-s-RNA formation was also the same for the two s-RNA species, in each case about one-third of that obtained when enhancing factor was also present (Table V). Thus, purified yeast enzymes appear to be able to form seryl-s-RNA with both of the *E. coli* serine s-RNA species. The difference in the results obtained by Bennett and his collaborators and by us have not yet been explained. Perhaps they are related to the level of purity of the heterologous enzyme used.

Discussion

The kinetic studies with cytidine- and adenosine-terminal serine s-RNA species reported here indicate that the terminal adenosine not only functions as the site of aminoacylation, but also provides structural features of major importance for binding of serine s-RNA to the enzyme complex. The absence of terminal adenosine in an otherwise intact serine s-RNA chain would appear to result in an inert s-RNA rather than a competitively inhibitory species. Torres-Gallardo and Kern (1965) have recently reported that *E. coli* s-RNA which has been degraded by periodate oxidation (which leaves the terminal base still intact) or by snake venom exonuclease digestion (which removes the terminal-adenosine and cytidine residues) competitively inhibited valyl- and tyrosyl-s-RNA formation with native s-RNA; however, kinetic analysis indicated that the inhibitory s-RNA had significantly lower affinity for the enzyme complex than did native s-RNA. Thus, the terminal adenosine of s-RNA appears to be of importance in the interaction not only of yeast serine s-RNA, but also in the interaction of *E. coli* valine and tyrosine s-RNA with the enzyme complex. The quantitative importance of the terminal adenosine of s-RNA in this interaction possibly will vary considerably for different amino acid specific s-RNA's and for s-RNA's from different species. The chemical basis for the observed kinetic differences between *E. coli* and yeast serine s-RNA's with the yeast enzyme is not known at present. The serine s-RNA's from these two species show quite similar behavior on Sephadex partition

TABLE V: Maximal Serine Incorporation into *E. coli* s-RNA-SerI and s-RNA-SerII.[a]

Enzyme Addn	μmoles of [^{14}C]Serine Incorpd/OD_{260} Unit of s-RNA[b]	
	s-RNA-SerI × 10^6	s-RNA-SerII × 10^6
E. coli enzyme[c]	92.7	792
Crystalline yeast seryl-s-RNA synthetase	21.7	223
Yeast seryl-s-RNA synthetase + yeast enhancing factor[d]	73.5	704

[a] The two *E. coli* serine s-RNA species were prepared by countercurrent distribution and kindly furnished by Drs. J. Goldstein, T. P. Bennett, and F. Lipmann of the Rockefeller Institute, New York, N. Y., [b] Absorbancy unit at 260 mμ. [c] The supernatant fraction of *E. coli* prepared following sonic disintegration of cells by high-speed centrifugation as described by Bennett *et al.* (1963). [d] The step 5, arginyl-s-RNA synthetase fraction (55% saturated ammonium sulfate extract) from yeast (Makman and Cantoni, 1965).

chromatography and their chemical compositions are similar in some respects; however, differences exist in over-all base composition and in the particular minor bases present in each. Serine s-RNA's from both species contain thymine riboside but only that from yeast contains dimethylguanosine (Cantoni *et al.*, 1963; Nathenson *et al.*, 1964). Peterkofsky (1964) has reported that the leucine-acceptor capacity of methyl-deficient *E. coli* s-RNA is decreased when measured with yeast leucyl-s-RNA synthetase, but is unaltered when measured with an *E. coli* enzyme preparation. Studies carried out by Dr. S. G. Nathenson[5] in our laboratory have indicated that the total acceptor capacity of *E. coli* s-RNA for serine measured with yeast enzyme is not altered when methyl-deficient s-RNA is used in place of normal s-RNA.

The kinetic studies reported here concerning seryl-s-RNA formation catalyzed by the purified yeast enzyme and with serine-specific s-RNA's from yeast and *E. coli* showed that the *E. coli* substrate had a lower affinity for the enzyme and also that the maximum velocity was lower with the *E. coli* substrate. The difference in maximum velocity indicates that the transfer reaction involving seryl adenylate–enzyme complex and heterologous s-RNA is less efficient than with the homologous substrate. The difference in V_{max} may be merely an apparent one, due to a greater lability of the *E. coli* seryl-s-RNA product (not yet ruled out). Also, the difference may be due to a slower rate of transfer of serine to heterologous s-RNA. Perhaps, however, with either s-RNA substrate present the acyl linkage of the seryl adenylate–enzyme complex is rendered labile, but that due to a "poor fit" of *E. coli* s-RNA on the complex only a fraction of the seryl residue can be transferred to *E. coli* s-RNA, while the rest is transferred to water. The striking effect of phosphate on the reaction with *E. coli* substrate may be due to a further enhancement of the transfer of serine from the complex to water. Relevant to this interpretation are the elegant studies of Norris and Berg (1964) with highly purified preparations of isoleucine s-RNA synthetase from *E. coli*. This enzyme catalyzes valyl as well as isoleucyl adenylate formation, but only isoleucine is transferred to s-RNA. Enzyme aminoacyl adenylate complexes with both valine and isoleucine were formed and isolated; addition of *E. coli* isoleucine s-RNA caused a breakdown of the enzyme–valyl adenylate complex, whereas isoleucine was transferred directly from the complex to the s-RNA. In these studies of Norris and Berg (1964), the amino acid component of the system has been varied, while in our studies the s-RNA has been varied; hence, the situations are only partially analogous.

Species specificity in the formation of aminoacyl-s-RNA has been investigated by a number of workers (Benzer and Weisblum, 1961; Berg *et al.*, 1962; Bennett *et al.*, 1963; Doctor and Mudd, 1963; Sueoka and Yamane, 1963; Allende and Allende, 1964; Lagerkvist and Waldenstrom, 1964; Peterkofsky, 1964). These studies generally have not taken into consideration either the relative proportion of adenosine-terminal s-RNA present in s-RNA preparations from different species, or the presence or absence of CCA-pyrophosphorylase in the enzyme preparations used. However, for some of these studies purified or partially purified enzymes have been used (Berg *et al.*, 1962; Clark and Eyzaguire, 1962; Lagerkvist and Waldenstrom, 1964; Peterkofsky, 1964) and rates as well as total extent of aminoacyl-s-RNA formation have been measured (Clark and Eyzaguire, 1962; Lagerkvist and Waldenstrom, 1964). Lagerkvist and Waldenstrom have made quantitative comparisons of rates with heterologous and homologous s-RNA using enzymes and s-RNA from yeast and *E. coli*.

Bennett *et al.* (1963) and Goldstein *et al.* (1964) have reported that yeast enzymes catalyze leucyl s-RNA formation with only two of the four *E. coli* leucine s-RNA species (separated by countercurrent distribution techniques) which are available to *E. coli* enzyme. Also, as mentioned earlier, Goldstein *et al.* (1964) have found that yeast enzymes catalyze seryl-s-RNA formation with only one of the two species of *E. coli* serine s-RNA which are available to *E. coli* enzyme. In contrast, we have reported here that both of the same two *E. coli* serine s-RNA species can be acylated with purified yeast enzymes. The extent of acylation of both of these *E. coli* s-RNA species depends on the presence of a

[5] S. G. Nathenson, unpublished observations.

heat-labile factor different from seryl-s-RNA synthetase.

The effect of the enhancing factor might not be limited to serine. Two leucine s-RNA peaks can be obtained by partition chromatography of the *E. coli* s-RNA. In agreement with Goldstein *et al.* (1964) we have observed that crude yeast enzymes could not acylate maximally one of the two peaks (peak A) while peak B responded to crude yeast enzyme as well as to *E. coli* enzyme. However, with partially purified leucyl-s-RNA synthetase both peaks can be charged maximally (82 and 88% of the valine obtained with *E. coli* s-RNA). Purified leucyl-s-RNA synthetase, because of the method of preparation, may well contain the so-called enhancing factor.

Finally, it should be mentioned that Boman and Svensson (1961) have reported on the presence of a "regenerating enzyme" in a preparation of arginyl-s-RNA synthetase from *E. coli* which was capable of enhancing the incorporation of methionine into *E. coli* s-RNA in the presence of yeast methionyl-s-RNA synthetase.

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