# AMINO ACID ACCEPTOR AND TRANSFER FUNCTIONS OF sRNA CONTAINING 8-AZAGUANINE

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The incorporation of 8-azaguanine into the RNA of Bacillus Cereus has been amply documented (Matthews and Smith, 1956; Mandel and Markham, 1958; Otaka et al, 1961, 1962; Levin, 1963a, 1963b; Grunberger and Sorm, 1963; Grunberger et al, 1964). An analysis of $sRNA_{azaG}$* has revealed that only guanine residues are replaced by the base analogue; the nucleotide sequence is not otherwise altered (Levin, 1963b). However, a decrease in the extent of secondary structure occurs as a result of azaguanine incorporation. The loss of secondary structure is proportional to the increase in analogue content and is attributed to the weak hydrogen-bonding properties of 8-azaguanine (Levin and Litt, 1964). In spite of the chemical and structural modifications induced in sRNA by the presence of azaguanine residues, isolated $sRNA_{azaG}$ can accept several amino acids to the same extent as $sRNA_N$. Moreover, $sRNA_{azaG}$ and $sRNA_N$ stimulate poly U-directed polyphenylalanine synthesis in a similar manner in B. cereus extracts.

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* Abbreviations: $sRNA_N$, B. cereus normal sRNA; $sRNA_{azaG}$, B. cereus azaguanine-containing sRNA; azaG, 8-azaguanosine 5′-P; azaGTP, 8-azaguanosine 5′-triphosphate; mRNA, messenger RNA; S30, 30,000 x g extract; S100, 105,000 x g extract; TCA, trichloroacetic acid.

## METHODS AND MATERIALS

Bacillus cereus cultures were grown in the presence of 8-azaguanine as previously described (Levin, 1963b). Normal cultures were grown in a similar manner except that the organisms were harvested at an $A_{540}$ of 0.75. Cell-free extracts were obtained by grinding the washed organisms with acid-washed Alumina A-305 (Alcoa). S30 and S100 extracts were prepared according to the method of Nirenberg and Matthaei (1961) and passed through Sephedex G-25 (0.9 cm x 30 cm) to remove small components. $sRNA_N$ and $sRNA_{azaG}$ were isolated by ethanol-KCl precipitation after phenol treatment of the appropriate DNase-treated S100 extract and purified by chromatography on DEAE-cellulose columns (Levin, 1963b). This procedure yielded sRNA fractions which displayed single peaks upon passage through columns of Sephedex G-25 and Sephedex G-200, respectively. E. coli and B. cereus S100 extracts served as the source of amino acid activating enzymes; endogenous sRNA was removed by passing 2cc of each extract through a DEAE-cellulose disc (1.8cm x 1cm). After washing the disc with 10 volumes of 0.05 M KCl, the active enzyme fractions were eluted with 0.3 M KCl. The effluent salt was removed by passing the extracts through Sephedex G-25 in the presence of 0.01 M Tris, pH 7.5-0.001 M EDTA-0.003 M glutathione-0.01 M KCl. Carbon-14 amino acids with specific activities of 5-10 mcuries per mmole were obtained from Nuclear-Chicago.

## RESULTS

Table I demonstrates that an $sRNA_{azaG}$ preparation in which 19% of the guanine residues were replaced by 8-azaguanine retained the capacity to accept L-leucine, L-lysine, L-alanine, and L-valine in the presence of B. cereus activating enzymes. The relative acceptor ability of $sRNA_{azaG}$ and $sRNA_N$ for a given amino acid was essentially

the same in all cases. All values represent averages of duplicate determinations except for valine where both values are given. In addition, B. cereus enzyme fractions obtained from separate growth cultures (I and II) were utilized in order to determine the reproducibility of this system. Though fraction II yielded slightly higher values for valine-$C^{14}$ incorporation than fraction I, the relative acceptor capacity of $sRNA_N$ and $sRNA_{azaG}$ remained the same.

TABLE I

Comparison of Amino Acid Acceptor Activity of $sRNA_{azaG}$ and $sRNA_N$

| Amino acid | Mole fraction of sRNA charged x 100 | | | |
|---|---|---|---|---|
| | B. cereus extract | | E.coli extract | |
| | $sRNA_N$ (%) | $sRNA_{azaG}$ (%) | $sRNA_N$ (%) | $sRNA_{azaG}$ (%) |
| L-Leucine | 3.3 | 3.1 | 4.0 | 4.1 |
| L-Lysine | 2.8 | 2.6 | 2.9 | 3.0 |
| L-Alanine | 1.6 | 1.7 | - | - |
| L-Valine - I | 3.3 | 3.2 | 3.5 | 3.4 |
| | 3.3 | 3.0 | - | - |
| L-Valine - II | 3.7 | 3.5 | - | - |
| | 3.8 | 3.5 | - | - |

The incubation mixtures contained the following components in a final volume of 0.1 ml: 0.05 M Tris-HCl, pH 7.4; 0.015 M $MgCl_2$; 0.005 M ATP; 0.0005 M CTP; 0.02 M KCl; 70 to 85 mμmoles of each amino acid (containing 0.3 to 0.5 μC of $C^{14}$ each); 33 μg of $SRNA_{azaG}$ or $sRNA_N$ as indicated; and 200 to 300 μg of DEAE-cellulose-treated extract protein of B. cereus or E. coli. These proportions represented a limiting amount of sRNA concentration in the presence of excess enzyme. The reaction was complete after 20 minutes incubation at 37°. The tubes were chilled and 0.9 ml of 6% TCA was added. The tubes were left standing in ice for 15 minutes. The suspensions were added to millipore filters (pore diam: 0.45 μ) and washed three times with 6% TCA. The filter membranes were placed in scintillation vials and counted in a dioxane-naphthalene-PPO-POPOP scinitillation solution in an ambient-temperature liquid scintillation counter (Nuclear-Chicago, model 723). Values are expressed as the per cent of total sRNA chains which accept a given amino acid, based on a molecular weight of approximately 26,000 for both $sRNA_N$ and $sRNA_{azaG}$ (unpublished data).

When E. coli extracts replaced B. cereus extracts as the source of activating enzymes, $sRNA_N$ and $sRNA_{azaG}$ again displayed the same relative amino acid acceptor capacities (Table I). The absolute values were slightly but consistently higher which might be attributed to the greater stability of the E. coli enzymes or alternatively to the presence of a nuclease-type activity in the B. cereus extracts which would tend to reduce acceptor ability. The low values obtained for the incorporation of alanine are as yet unexplained.

Figure 1 shows that S30 extracts prepared from normal B. cereus cultures catalyze the formation of poly U-directed polyphenylalanine.

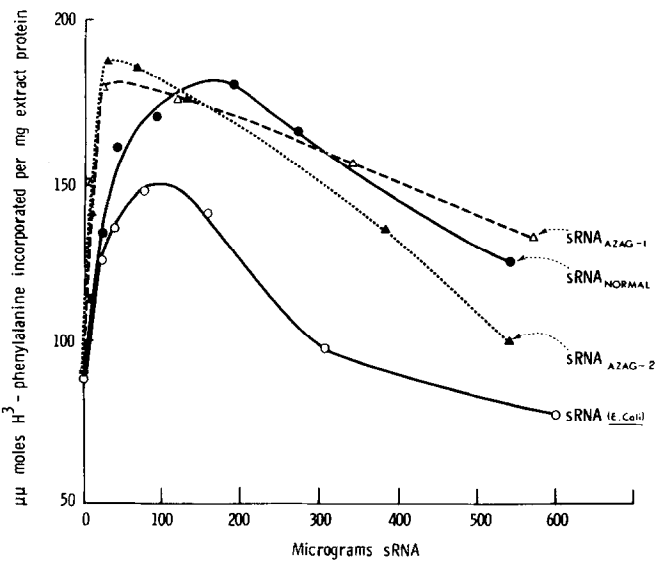


Figure 1. Effect of $sRNA_N$ and $sRNA_{azaG}$ on polyphenylalanine synthesis in normal B. cereus extracts. Each point was determined from duplicate incubation mixtures containing the following components in a final volume of 0.25ml: 0.1 M Tris-HCl, pH 7.9; 0.022 M $MgCl_2$; 0.096 M KCl; 0.006 M $\beta$-mercaptoethanol; 0.004 M PEP; 0.001 M ATP; 0.0002 M GTP; 25 mµmoles $H^3$-phenylalanine containing 0.12 µcurie (Nuclear-Chicago); 20 µg of PEPkinase; and 400 µg of B. cereus extract protein. Incubation was at 37° for 15 minutes. The reaction was stopped by the addition of 0.75 ml of 7.5% TCA heated at 90° for 15 minutes and precipitable radioactivity was determined as described in Table I. The curve of $sRNA_N$ is an average of 2 separate $sRNA_N$ batches; $sRNA_{azaG-1}$ contains a 19% replacement of G by azaG; $sRNA_{azaG-2}$ contains a 29% replacement of G by azaG.

The addition of $sRNA_N$ or $sRNA_{azaG}$ stimulated polyphenylalanine synthesis in a similar manner. At low concentrations $sRNA_{azaG}$ appeared to be slightly more effective. At high concentrations, both $sRNA_N$ and $sRNA_{azaG}$ produced an inhibition of polyphenylalanine synthesis. This phenomenon has been previously described and attributed to a preferential binding of certain amino acyl-sRNA chains to the mRNA-ribosome complex (Grunberg-Manago and Dondon, 1965; Leder and Nirenberg, 1964). Since the sRNA content per cell rises rapidly in azaguanine cultures this may account in part for the inhibition of protein synthesis observed *in vivo* in these cultures (Levin, 1963b).

*E. coli* sRNA (General Biochemicals) was less active in promoting polyphenylalanine synthesis in the *B. cereus* system (Fig. 1). The reduced activity may be a reflection of inefficient charging by *B. cereus* enzymes. This is in contrast to the efficiency of *E. coli* enzymes in charging *B. cereus* $sRNA_N$ and $sRNA_{azaG}$ (Table I), an observation which has recently been corroborated by other investigators (Weinstein and Grunberger, 1965).

## DISCUSSION

The results obtained in the present study indicate that $sRNA_{azaG}$ can accept the amino acids leucine, lysine, alanine, and valine. The evidence also suggests that phenylalanine is accepted by $sRNA_{azaG}$ and transferred to ribosomes in the course of polyphenylalanine synthesis in *B. cereus* extracts. In support of this observation, other studies indicate that *B. cereus* $sRNA_{azaG}$ and $sRNA_N$ stimulate polyphenylalanine synthesis in *E. coli* extracts to the same degree; miscoding anomalies which might arise from the presence of azaguanine residues in sRNA were not detected for those amino acids examined (Weinstein and Grunberger, 1965). It is clear, however, that more rigorous evidence based on the ability of specific $sRNA_{azaG}$ chains to accept a given amino

acid and transfer it to polypeptides is required.

It should be emphasized that $sRNA_{azaG}$ contains a high proportion of $sRNA_N$ chains. An $sRNA_{azaG}$ batch in which 20% of the total guanine residues have been replaced by 8-azaguanine may contain as much as 60% $sRNA_N$ chains which are present in the B. cereus culture prior to azaguanine addition. Subsequent sRNA synthesis results in the formation of $sRNA_{azaG}$ chains with increasing amounts of azaG per chain. Previous studies indicate that the distribution of azaG in these chains appears to be random (Levin, 1963b). It is conceivable, however, that some G sites in a given chain are more susceptible to replacement by azaG than others. This reservation must be considered in evaluating the results.

Further data from several sources make it clear that azaG in RNA behaves functionally like G: a) azaG bonds in Poly azaG and $sRNA_{azaG}$ are susceptible to enzymic hydrolysis by takadiastase-$T_1$-ribonuclease (Levin, 1962, 1963b); b) azaGTP replaces GTP as a substrate in DNA-primed RNA synthesis catalyzed by RNA polymerase (Kahan and Hurwitz, 1962); c) azaG replaces only G during the course of in vivo synthesis of $sRNA_{azaG}$ (Levin, 1963b); and d) synthetic azaGU polymers of different azaG:U ratios stimulate the incorporation of specific amino acids into polypeptides in a manner similar to UG polymers of comparable ratios (Grunberger et al, 1965). In addition, recent studies indicate that B. cereus extracts prepared from azaG cultures are capable of catalyzing the synthesis of polypeptides utilizing endogenous mRNA containing azaguanine residues (Grunberger, 1965; Levin, manuscript in preparation).

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