THE INCORPORATION OF AMINO ACIDS INTO "METHYL-POOR" AMINO ACID TRANSFER RIBONUCLEIC ACID*

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In the preceding paper (Starr, 1962) the presence of methyl groups primarily in soluble or amino acid transfer RNA (S-RNA) rather than in another non-particulate RNA species was confirmed. Methyl groups were also noted to be associated with ribosomal RNA, but this is of no significance to the current experiments. The question was subsequently raised whether the pattern of methylation plays a role in the amino acid specificity of the S-RNA or whether it imparts structural, non-coding (in the genetic sense) modifications to the molecule.

METHODS

Normal and "methyl-poor" RNA were prepared and assayed as described in the preceding paper (Starr, 1962). A mixture of amino acid activating enzymes was made by grinding E.coli B with 3 times their weight of Alumina followed by extraction with 3 volumes of buffer solution containing 0.01M MgAcetate, 0.06M KCl, 0.006M Mercapto-ethanol and 0.01M Tris buffer, pH 7.6. The residue was removed by centrifugation for one hour at 30,000 xG at 0°C. DNase was added to the supernatant solution at a concentration of 3 µgm/ml and the mixture was incubated at 0°C for 15 minutes. The solution was then centrifuged at 105,000 xG for 2 hours. The resulting supernatant solution was stored at -20°C in small aliquots for use as the crude enzyme preparation.

Amino acid incorporation was determined in incubation mixtures containing crude activating enzyme solution 0.05 ml, RNA 0.1 mgm, amino acid 0.05 µmoles, (2 µc/µmole),

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$MgCl_2$ 1.0 µmole, pyruvic kinase 3 µgm, PEP 1.0 µmole, ATP 0.4 µmole, Tris pH 7.4, 10.0 µmoles, in a volume of 0.2 ml. Incubation time, unless otherwise stated, was one hour, in order to allow maximum amino acid incorporation. C^{14}-amino acid incorporation into RNA was analyzed by a paper disc method described elsewhere (Starr and Goldthwait, 1962). Radioactivity was determined by immersing the washed and dried disc directly in scintillation liquid and counting in a Tri-Carb liquid scintillation spectrometer.

RESULTS

Incorporation of amino acids into normal and "methyl-poor" S-RNA: The incorporation of nine amino acids into each kind of S-RNA is reported in Table I. It will be noted that with all the amino acids tested, incorporation into both types of S-RNA was essentially comparable. Approximately one half of the "methyl-poor" S-RNA was formed prior to the methionine starvation (Starr, 1962) and was, therefore, normally methylated. This could account for some of the amino acid incorporation. If the "methyl-free" S-RNA synthesized after methionine starvation were incapable of accepting amino acids, however, a difference of about 50% would be expected between normal and "methyl-poor" S-RNA. In no instance was the difference greater than 20% (arginine), and the difference is occasionally in favor of the "abnormal" RNA.

Table I

Amino acid incorporation into normal and "methyl-poor" S-RNA

Amino Acid	Normal S-RNA CPM/mgm	Methyl-poor S-RNA CPM/mgm
Alanine	970	960
Arginine	3450	2800
Glutamine	4000	3900
Glycine	1440	1450
Leucine	1950	2100
Lysine	1650	1700
Phenylalanine	880	700
Tyrosine	1000	980
Valine	1740	1700

C^{14}-Leucine incorporation into increasing amounts of S-RNA: The incorporation of leucine using increasing amounts of normal and methyl-poor S-RNA during a one hour incubation is reported in Table II. The increases in incorporation into both types of S-RNA were roughly proportional and in parallel.

Table II

Leucine incorporation into increasing amounts of S-RNA

mgm S-RNA added	Normal S-RNA CPM	Methyl-poor S-RNA CPM
0	0	17
0.025	44	66
0.05	129	130
0.10	233	232
0.15	280	315
0.20	505	460

Incubation mixtures were as described in the text, except for the amount of RNA added. Incubation was for one hour at 37°C.

Kinetics of incorporation: The kinetics of C^{14}-leucine incorporation with respect to time are illustrated in Figure I. It may be noted that the rates of incorpo

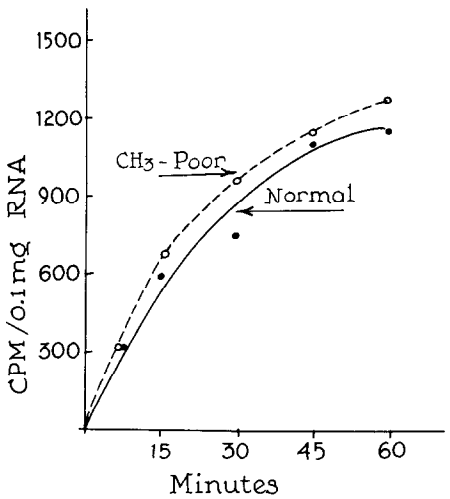

Figure I

Incorporation of C^{14}-Leucine into normal and "methyl-poor" S-RNA with respect to time. Conditions of incubation are described in the text.

ration into normal S-RNA and methyl-poor S-RNA are essentially identical. In another experiment, the rates of incorporation of C^{14}-leucine into methyl-poor S-RNA and methyl-poor S-RNA partially remethylated *in vitro* by RNA methylase, were similar.

DISCUSSION

The existence of a specific amino acid transfer RNA for each amino acid has long been known (Berg and Ofengand, 1958). The role of S-RNA in amino acid coding and the existence of several S-RNA species for one amino acid have recently been demonstrated (Chapeville et al, 1962; Weisblum et al, 1962). It has always been assumed that the coding of the S-RNA is intrinsic in the nucleotide sequence of the molecule. The number of nucleotides involved and the site along the chain are unknown. Although a triplet code analogous to that of messenger RNA might be involved in the transfer of amino acids to the ribosomal template, there is nothing yet available to indicate the requirements for coding for amino acid acceptance, nor whether the coding sites for the two functions are identical. The amino acid itself is not involved in transfer specificity (Chapeville et al, 1962). Whether the base-sequence of each S-RNA molecule is identical except at small coding areas is unknown. Berg et al, (1962) have demonstrated differences among the (acceptor) terminal sequences of isoleucine-specific RNA and two leucine-specific RNAs.

The methylated bases which are found in trace amounts do not randomly replace their unmethylated analogues along the nucleotide chain (Ingram and Pierce, 1962), but tend to be clustered around the center of the molecule (Nihei and Cantoni, 1962). The methylated bases apparently are not necessary for the amino acid acceptor function of S-RNA, a finding which could be explained by the absence of methylated bases in the area which determines acceptor specificity. This site may be located in the terminal positions of the molecule, possibly near the amino acid acceptor site.

Spencer (1962) has postulated that the coding site of amino acid transfer specificity resides in a non-hydrogen bonded hair-pin bend which occurs in the middle of an otherwise double helical, rigid, assymmetrical structure (Brown and Zubay, 1960;

Spencer et al, 1962; Luborsky and Cantoni, 1962). Since the methylated bases, which would form H-bonds poorly in the double helix, occur primarily in mid-molecule, Zamecnik (1962) has suggested that they may be found in or near the hair-pin bend and may play a role in the transfer function of S-RNA. This role may be either in the coding itself or in modification of the physical structure and hydrogen-bonding capacity of S-RNA at the site of binding to template RNA. The transfer activity of methyl-poor RNA is currently under investigation.

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