EFFECTS OF ULTRAVIOLET LIGHT ON THE BIOLOGICAL FUNCTIONS OF TRANSFER RNA*

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Received January 12, 1966

We (Scott and Turter, 1962; Buc and Scott, 1965) and others (Fawaz-Estrup and Setlow, 1964; Zachau, 1964; Swenson and Nishimura, 1964; Fukutome et al., 1964; Kawade et al., 1965; Wacker et al., 1964; Gottschling and Zachau, 1965) have reported on the effects of ultraviolet light on the capacity of transfer RNA (t-RNA) from yeast or from E. coli to accept certain amino acids from the aminoacyl ligases and to transfer amino acids into polypeptide linkage. We wish here to report on the comparative effect on the acceptance capacity of t-RNA and on t-RNA already charged with ^{14}C amino acids to: (a) bind non-enzymatically to the ribosome:artificial messenger complex and (b) to participate in the complete enzymatic system for the formation of polypeptide.

Yeast t-RNA was isolated by the method of Monier (1960) from baker's yeast. That from E. coli was purchased. Solutions of t-RNA, approximately 3 mg/ml in 0.15 M NaCl 0.015 M sodium citrate pH 7, were stirred rapidly during exposure to the flux of a low pressure Hg arc which passed a filter of 4 cm of absolute ethanol. The photochemically active flux was estimated at the beginning and end of the period of

* This work was supported by grants from the American Cancer Society and the United States Public Health Service (C-558) and by AEC Contract AT(30-1)-2643. One of us (M.-H.B.) was supported, in part, by le Centre National de la Recherche Scientifique. This is publication No. 1242 of the Cancer Commission of Harvard University.

radiation by means of a uridine actinometer using a quantum yield of 0.0216 (Sinsheimer, 1954). The quantum yields reported here were calculated from the dose of radiation necessary to produce an average of one biologically effective hit per t-RNA molecule (dose to reduce the acceptance to 1/e of the control level). This dose (einsteins/cm^2) was then divided into the moles of t-RNA absorbing the dose. Molecular weights of 28,000 and 30,000 were used for t-RNA from yeast and E. coli respectively. The reported doses and calculated quantum yields are, therefore, relative to uridine and do not represent absolute values.

The assay for acceptance capacity was carried out by a membrane filtration technique (Bergquist and Scott, 1964) using semi-purified, homologous aminoacyl ligase preparations. The assay conditions yielded stable plateau values for acceptance in the case of both RNA's. Assays for non-enzymatic binding were performed by the method of Leder and Nirenberg, as modified by R. Thach (1965). The polynucleotide-directed polypeptide synthesis was assayed by a membrane filtration technique devised by Lamborg (personal communication). In both cases t-RNA was charged under standard conditions prior to irradiation. Samples were taken during the irradiation to determine the rate of loss of label and to provide correction factors for the amount of ^{14}C amino acid transferred or bound.

RESULTS

Figure 1 presents graphically an example of the comparative sensitivity of phenylalanine acceptance capacity of t-RNA from E. coli and from yeast. It can be seen that the results are consistent in both instances with first-order kinetics, that there is evidence of two slopes for t-RNA from coli, and that there is considerable difference in the relative sensitivities of the t-RNA from these two species (the ratio of slopes is approximately 11). Among those amino acids tested, the ratio of initial slopes varies over approximately a 20-fold range

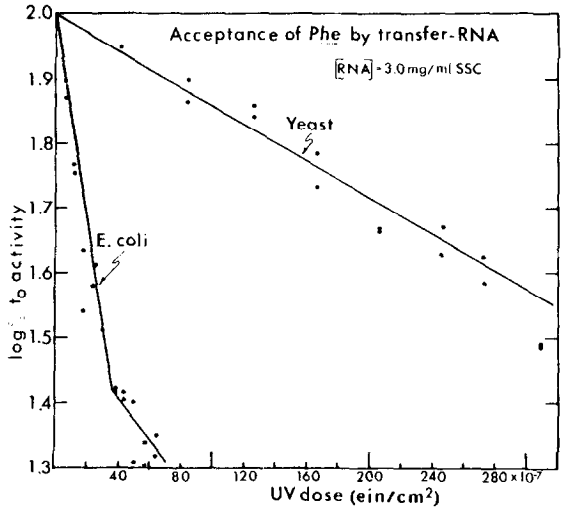

Fig. 1 A comparison of the loss of acceptance capacity of t-RNA from yeast and from E. coli for phenylalanine as a function of the dose of ultraviolet light. The amount of ultraviolet light absorbed by the samples in this and subsequent figures is expressed as einsteins/cm^2 measured as described in the text.

(Table 1). The range of relative sensitivities within each species is not greatly different, but t-RNA from coli is, for a given amino acid, always more sensitive than that from yeast.

Table 1

Apparent Quantum Yield
(loss of acceptance capacity)

Amino acid	E. coli t-RNA	Yeast t-RNA	Ratio
Leu	5.7×10^{-3}	-	-
Ilu	7.0	4.0×10^{-3}	1.8
Ser	12	-	-
Asp	12	1.1	11
His	14	-	-
Tyr	15	-	-
Gly	18	-	-
Thr	26	-	-
Ala	37	3.4	11
Arg	43	1.0	43
Lys	42-52	3.3	14
Phe	36-51	4.2	10
Val	53	1.4	38
Pro	61	-	-

We have observed two slope kinetics in t-RNA from E. coli and for the

following amino acids: Phenylalanine, lysine, valine, alanine, arginine, and probably for glycine, glutamic acid, and leucine. We shall show in a separate paper that this phenomenon is related, at least in part, to the existence of multiple acceptors for a single amino acid.

We thought it of interest to compare the relative sensitivities of t-RNA for the acceptance of amino acids with those for the non-enzymatic binding to the artificial messenger:ribosome complex, and for the enzymatic formation of homopolypeptides directed by synthetic polynucleotides (Figures 2 and 3). The most immediate inference from Figure 2 is of the very large difference in the sensitivity of t-RNA for the acceptance step and for the non-enzymatic binding step. The phenylalanyl acceptor molecule is essentially insensitive to ultraviolet light for this latter function in the dose range covered (sufficient to reduce the acceptance to less than 5% of the control level). This insensitivity is consistent

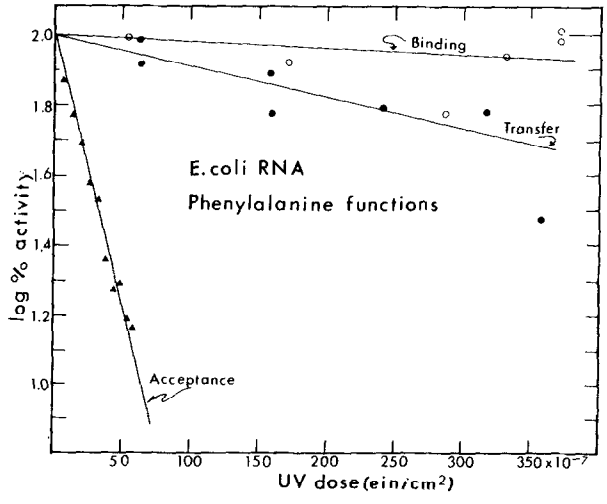

Fig. 2 Comparison of the relative sensitivities on t-RNA from E. coli for three functions of t-RNA: (1) acceptance of the amino acid (in this case, phenylalanine) from the aminoacyl ligase, (2) the non-enzymatic binding of the charged t-RNA to the messenger:ribosome complex, and (3) the enzymatic transfer of the amino acid from t-RNA to polypeptide linkage.

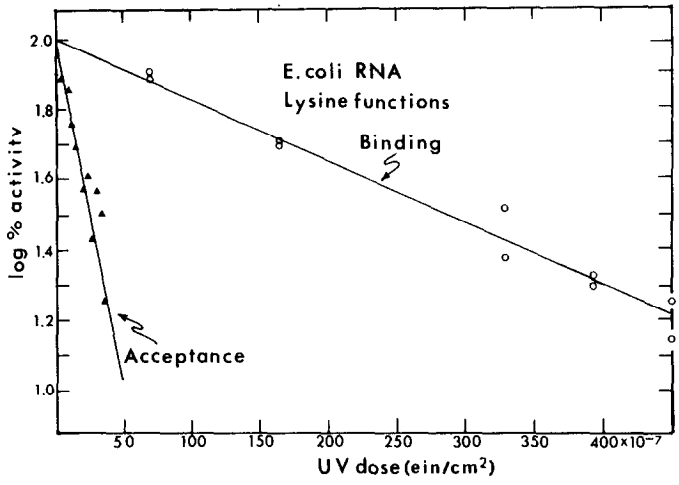

Fig. 3 Comparison of the sensitivities of acceptance and of binding capacities for lysine of t-RNA from E. coli.

with the high resistance to ultraviolet light of AMP residues in t-RNA and the restricted involvement of the putative anticodon for phenylalanine in the non-enzymatic binding of t-RNA to the poly U:ribosome complex. The significant sensitivity of charged t-RNA to ultraviolet light for the enzymatic transfer of phenylalanine is interesting in this regard, as it implies a necessary interaction between the t-RNA molecule and one or more of the enzymes involved in the peptide bond formation, which interaction is sensitive to alteration of the structure of the RNA molecule. The foregoing result is in contrast to the significant sensitivity of lysine-charged t-RNA for the same function. Again, this is consistent with the known UV sensitivity of UMP residues in t-RNA and the putative anticodons for lysine (UUU, UUC, AUU) (Leder et al., 1965).

DISCUSSION

The difference between the UV sensitivities of the t-RNA's of yeast and E. coli for the capacity to accept amino acids from the amino-acyl ligases is quite remarkable. There is little in the hyperchromicity

data, the optical rotatory dispersion (Lamborg et al., 1965), the base ratios, or the frequency runs of pyrimidines (Bergquist and Scott, 1964) which suggest an explanation for this difference. The possibility that the aminoacyl ligases from E. coli may require a higher precision of structure than do the enzymes from yeast or mammalian tissue is now being examined using common heterologous ligases.

If one accepts the argument that the high resistance to UV light of t-RNA charged with phenylalanine with respect to its capacity to bind to the ribosome:poly U complex is evidence supporting the participation of the anticodon in this binding, then certain inferences from the lysine data (Figure 3) are possible. It is reasonable to postulate that photochemical damage to one U residue in the anticodon is sufficient to abolish the binding capacity for the t-RNA molecule. The ratio of the slopes (acceptance/binding) is approximately 12. If the calculated inactivation cross section for the binding function represents two nucleotides (i.e., either of two UMP residues), one would then estimate that the cross section for acceptance represents 24 nucleotides. If only the pyrimidines are involved in the photochemical damage, this would represent approximately 24/41sts (Bergquist and Scott, 1964) of the entire molecule of 86 nucleotides, or 60% of the pyrimidines. There is little evidence concerning the frequency of clustering of pyrimidines in various t-RNA's but any reasonable extrapolation of existing data suggests that a photochemical alteration anywhere within a portion of the molecule equivalent to 1/3 to 2/3 of the whole was sufficient to inactivate the E. coli t-RNA molecule for the acceptance function. These conclusions are consonant with the results of others who have studied the effect of chemical (Yu and Zamecnik, 1963) and enzymatic (Nishimura and Novelli, 1964) modification of t-RNA on the acceptance capacity.

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