THE EARLY CHANGE IN E. COLI LEUCINE tRNA AFTER INFECTION WITH BACTERIOPHAGE T2*

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The chromatographic profile of Escherichia coli leucine tRNA is markedly altered after T2 infection (see Sueoka and Kano-Sueoka, 1964; Kano-Sueoka and Sueoka, 1966; Waters and Novelli, 1967). An early change involves changes in the quantity of the two major peaks observed by methylated albumin kieselguhr (MAK) or the three main peaks observed by reversed-phase column (RPC) chromatography and is completed within the first six minutes of infection. A later change involves the appearance of one or more leucine-tRNAs in the infected cells which are undetectable in normal or 6-minute infected cells (Waters and Novelli, 1967). This report is concerned with the nature of the alteration producing the early change.

Sueoka and Kano-Sueoka (1964 and 1966) have suggested that the early change results from a modification of the first main peak, as eluted off MAK, which causes it to elute in the position of the second peak. This suggestion is based on the following observations: 1) After phage infection, yeast synthetases charge the second peak of leucine tRNA whereas before infection the yeast enzyme apparently recognized only the first peak. 2) The total leucine acceptor activity did not change, relative to that of several other amino acids, after phage infection. Although our recent work has been directed primarily toward determining the origin of the new leucine tRNAs which are seen late in infection, we have accumulated evidence which suggests that the early change is the result of an apparently specific inactivation of the major leucine isoaccepting tRNA.

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METHODS

tRNA and aminoacyl-tRNA synthetase preparation. E. coli B cells were grown to a concentration of 5×10^8 cells/ml in the M-9 medium described by Adams (1959) supplemented with 0.05% casamino acids. Infection was done at the one liter scale as previously reported (Waters and Novelli, 1967). Isolation of tRNA was done by the method of Zubay (1962) as modified by Kelmers et al. (1965), except that the isopropanol step was omitted and a 1 M NaCl step was used to remove ribosomal RNA prior to DEAE column chromatography. In addition to the appropriate NaCl concentration, all aqueous solutions were buffered with 0.01 M Tris-HCl, pH 7.5, and contained 0.01 M $MgCl_2$, 0.001 M EDTA and 0.001 M Cleland's reagent.

Crude synthetases were prepared from normal cells as previously described (Kelmers et al., 1965), with the following additional steps. The ammonium sulfate precipitate was dissolved in 0.01 M KPO_4 buffer at pH 7.5 containing 0.01 M $MgCl_2$, 0.005 M KCl, 0.001 M glutathione, and 10% glycerol and passed over G-100 Sephadex equilibrated with the same solution. The main protein peak containing synthetase activity was then further purified by passage over DEAE-cellulose using the method of Muench and Berg (1966) as modified by Yang and Novelli (1968a). The fractions off DEAE containing synthetase activity were pooled and concentrated with 75% ammonium sulfate, desalted by passage through G-25 Sephadex equilibrated in 0.01 M Tris-HCl pH 7.5, 0.01 M $MgCl_2$, 0.001 M glutathione and 20% glycerol, and stored at -20°C in one to two ml aliquots. The G-100 Sephadex and DEAE-cellulose steps were required to eliminate nuclease-like activity which was present in the initial ammonium sulfate fraction.

Aminoacylation of tRNA. tRNA was aminoacylated in a final reaction volume of 0.25 ml containing per ml: Tris-HCl, pH 7.5, 100 µmoles; ATP, 4 µmoles; Mg acetate, 40 µmoles; KCl, 5 µmoles; β-mercaptoethanol, 1 µmole; ^{14}C amino acid, 4 µc (10.9-50.0 mµmoles); 1.52 to 3.24 $O.D._{260}$ units of mixed tRNA; and enough crude synthetase protein to completely charge the tRNA. Kinetics of charging were followed by pipeting 50 µl aliquots onto filter paper disks (Bollum, 1959), at timed intervals as described by Mans and Novelli (1961). Samples were counted in a liquid scintillation counter at an efficiency of approximately 50%. Under these conditions, incubation longer than 90 minutes yielded no net increase in charging with any of the amino acids tested. In all cases, the extent of charging was directly proportional to the amount of tRNA added. Samples for column chromatography were charged in 1 to 1.5 ml volumes (with either ^{14}C or ^{3}H leucine), and the aminoacyl-tRNA was

isolated by the DEAE-cellulose column procedure described by Yang and Novelli (1968b).

Column chromatography. Because of its increased resolving power, the RPC-2 (Freon) chromatographic system of Weiss and Kelmers (1967) was used instead of the RPC-1 (isoamyl acetate) previously used (Waters and Novelli, 1967). The RPC-2 system resolves normal E. coli leucine-tRNA into five components rather than the two observed on MAK columns or the three separated by RPC-1 chromatography. Mixed samples of ^{3}H and ^{14}C leucyl-tRNA were applied to the column at the NaCl concentration of the starting buffer and eluted using a 2 liter NaCl gradient containing 0.01 M Na acetate, pH 4.5; 0.01 M $MgCl_2$; 0.001 M EDTA (Na_2) and 0.005 M β-mercaptoethanol. Columns were run at the rate of 1.5 ml/min at 23°C. 10-ml fractions were collected in a refrigerated fraction collector. The labeled aminoacyl-tRNA was precipitated from each fraction by the addition of 2 $O.D._{260}$ units of calf thymus DNA (0.5 ml) and 2 ml of 50% TCA. The fractions were mixed by bubbling air through them. The nucleic acid was collected on Millipore filters, washed with 70% ethanol, dried and counted in a liquid scintillation counter.

RESULTS

Table 1 shows the total amino acid acceptor activity of normal and 6-min T2-infected E. coli tRNA for seven different amino acids. It is obvious that, when compared to the other six amino acids, leucine charging is less with 6-min T2 tRNA than with normal. This 6-min T2 tRNA preparation accepts, on the average, 11% more of the six amino acids (less leucine) than normal. If this increase is accepted as merely reflecting a measure of relative tRNA purity, normalizing to 100% reveals that 6-min T2 tRNA accepts leucine only 71% as well as normal tRNA. This decrease in leucine acceptor activity is characteristic of all 6-min T2 preparations tested.

If one fractionates equivalent amounts of normal and 6-min T2-tRNA in terms of radioactive amino acid on RPC-2, the results are as shown in Figure 1, curves A and B. It is obvious that if equal charging of both normal and 6-min T2 tRNAs were the case, and modification has occurred, then peak I has been converted into at least four other peaks. We feel that this is highly improbable. However, when the data are normalized to the actual charging results, i.e., 6-min T2 tRNA accepts leucine only 71% as well as normal, curve C results. When analyzed in this manner, it is clear that the early change in leucine tRNA after T2 infection involves only one of the major isoaccepting species, Ia. Analysis of the curves on a Dupont 310 curve resolver

TABLE 1

Amino Acid ^{14}C*	tRNA Sample	Specific Activity cpm/O.D.$_{260}$	% of Normal
Leucine	Normal	40,714	-
	6' T2	32,263	79
Valine	Normal	23,153	-
	6' T2	26,434	114
Lysine	Normal	10,727	-
	6' T2	11,500	107
Serine	Normal	13,116	-
	6' T2	13,211	101
Phenylalanine	Normal	20,874	-
	6' T2	24,342	116
Glycine	Normal	6,355	-
	6' T2	7,408	116
Methionine	Normal	13,017	-
	6' T2	14,921	114

*The specific activities of the amino acids were: leucine, 251; valine, 208.5; lysine, 150; serine, 122; phenylalanine, 366; glycine, 80 and methionine, 215 μc/μm.

shows that the 29% loss in charging activity can be completely accounted for by the decrease in peak Ia. Thus it appears that the altered chromatographic profile of leucine-tRNA, as observed after a 6-minute T2 infection on RPC-2, is primarily the result of an apparently specific inactivation of the isoaccepting species that is designated Ia. Results using yeast synthetases support this conclusion. The yeast enzyme charges peaks Ia, Ib and II of normal tRNA. After 6 minutes of T2 infection, a marked decrease in the charging of peak Ia without effect on Ib and II was observed.

Although it seemed unlikely that the inactivation of peak Ia after T2 infection was due to a selective loss of the -pCpCpA termini, experiments were done to eliminate this possibility. The -pCpCpA termini of both normal and 6-min T2 tRNA preparations

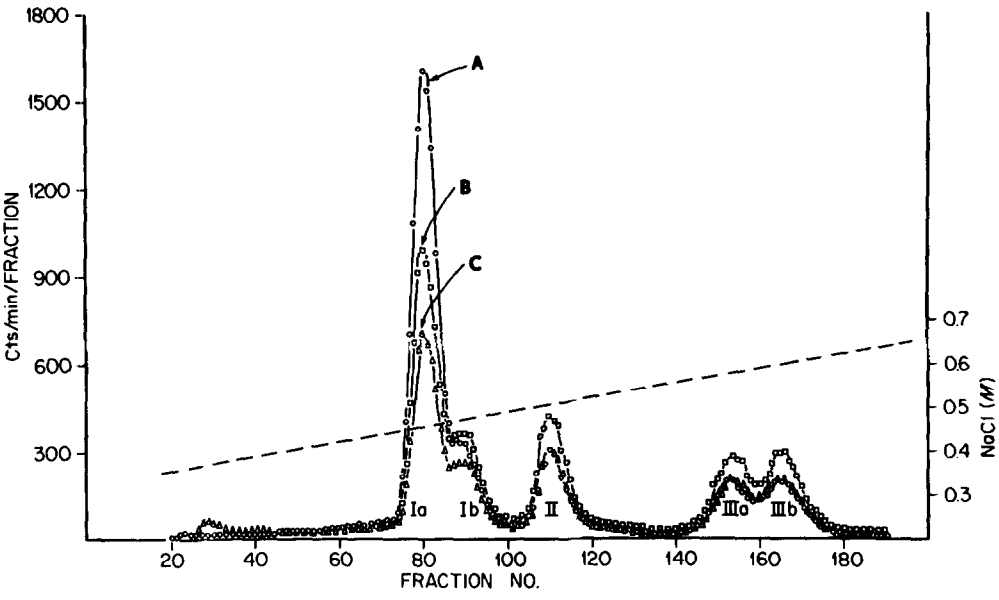

Figure 1. A. Normal E. coli Leucine-tRNA (^{3}H-21,200 cpm). B. 6-min T2 leucine-tRNA (^{14}C-21,200 cpm). C. 6-min T2 leucine-tRNA (^{14}C-15,000 cpm).

were damaged to about the same extent (about 10%), as determined by ^{14}C ATP incorporation in the presence of CTP. But although the crude synthetase preparation used had adequate -pCpCpA tRNA-adenylyl transferase (EC-2.7.7.20) activity to repair the damaged tRNA, addition of CTP to the reaction did not produce a detectable increase in the level of leucine acceptor activity in either the normal or 6-min T2 tRNA preparation. In addition the inclusion of CTP in the charging reaction did not alter the chromatographic profile of 6-min T2 tRNA.

Preliminary attempts to mimic, in vitro, the early leucine tRNA change by treating normal tRNA with crude, nucleic acid-free protein extracts from infected cells have thus far been unsuccessful.

DISCUSSION

We have shown that the altered chromatographic profile of E. coli leucine tRNA observed after a 6 minute T2 infection is the result of an apparently specific inactivation of the major isoaccepting species found in normal cells. The data do not support the possibility that one component has been modified in such a way as to chromatograph in the position of one or more of the other components. Except for the

discrepancy in the charging data, we believe that all the observations of Sueoka and Sueoka are consistent with our conclusion.

The cause of this inactivation is not clear. It does appear to be phage specific (see Kano-Sueoka and Sueoka, 1966). The fact that possibly only one amino acid (Sueoka and Kano-Sueoka, 1964) and most probably only one of its isoaccepting species (of which there are at least 5) is affected makes it unlikely to be artifactual. A possibility is that a phage-specific nuclease is responsible for the change. This might explain the front component (Leu F) described by Kano-Sueoka and Sueoka (1966), which eluted early off MAK and did not recharge after discharging, in that it might be a degradation product of peak Ia. Molecular weight estimates (Sueoka, personal communication) on this component support this idea. However, such a nuclease would show a degree of specificity not yet demonstrated in the known nucleases.

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