# ALTERED CHROMATOGRAPHIC PROPERTIES OF tRNA FROM CHLORAMPHENICOL-TREATED ESCHERICHIA COLI*

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SUMMARY

Several tRNAs from E. coli which had been treated with chloramphenicol (CAP) are shown to have altered chromatographic properties. The tRNA synthesized after CAP treatment is altered, whereas that already present in normal cells is not. Preliminary experiments indicate that the new aminoacyl-tRNAs which we observe are not results of undermethylation nor degradation.

Concentrations of CAP which cause essentially complete cessation of protein synthesis in E. coli also markedly reduce the rate of ribosomal RNA synthesis (Kurland and Maaloe, 1962). However, under these same conditions, the synthesis of tRNA continues (Kurland and Maaloe, 1962; Ezekiel and Valulis, 1965). Ezekiel and Valulis (1965) also showed that the tRNA which is synthesized in the presence of CAP is functional in that it can accept amino acids.

The present studies, originally initiated to test the effect of CAP on the appearance of the new leucine tRNA peaks detected in E. coli after phage infection (Waters and Novelli, 1967), confirm that functional tRNA is synthesized in normal E. coli in the presence of concentrations of CAP which prevent growth. Moreover, the chromatographic properties of the tRNAs are markedly altered. It is conceivable that the different

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isoaccepting species of tRNA which appear after CAP treatment are intermediates in the maturation process of tRNA and, as such, could prove useful in elucidating the steps involved in the synthesis of tRNA.

## METHODS

Growth and CAP treatment of E. coli. E. coli B cells were grown in New Brunswick jars, with continuous aeration at 37°C, in M-9 medium (Adams, 1959) supplemented with 0.05% casamino acids. The generation time was 35-40 minutes. When the cells reached a concentration of $5 \times 10^8$ cells/ml, either they were collected and used as the control sample or CAP was added to the culture at a final concentration of 50 μg/ml and the cells were harvested at various times thereafter.

Aminoacyl-tRNA synthetase and tRNA preparation. Crude synthetases were prepared from normal E. coli B, and tRNA was prepared from both normal and CAP-treated cells as previously described (Waters and Novelli, 1968).

Aminoacylation of tRNA. The conditions used to determine maximum acceptor activity of a tRNA preparation and for charging the tRNA for column chromatography were identical to those already described (Waters and Novelli, 1968).

Column Chromatography. Leucyl- and phenylalanyl-tRNAs were resolved using the RPC-2 (Freon) chromatographic system of Weiss and Kelmers (1967). We have recently improved our resolution capacity in this system by making certain that the prescribed amount of Freon is added to the packing. Because of the volatile nature of the Freon, the usual drip method of coating the chromasorb has resulted in a wide range of Freon, which is actually on the final packing. We find we can make a more reproducible packing by coating the packing very rapidly, e.g., the amine in Freon solution required for 150 gm of chromasorb is added within 15 minutes. Even under these conditions we routinely add an additional 5 ml of Freon per 56 ml of the amine solution to replace

the loss of Freon by evaporation. Using these technical modifications, we can achieve the desired weight-to-weight ratio (of amine in Freon to chromasorb) of 1:1. Packings with a low ratio of Freon to chromasorb are characterized by broader peaks. The columns were run and the fractions processed in the manner already described (Waters and Novelli, 1968), except that a column temperature of 20°C was used.

In the chromatographs shown, the data are normalized such that the area under the curve represents the relative amount of tRNA in the same weight of cells under the different physiological conditions.

## RESULTS AND DISCUSSION

Table 1 shows the increase in tRNA, with time, after exposure of E. coli to growth-inhibiting concentrations of CAP. That the specific acceptor activity of the tRNA, even after 4 hours of CAP treatment, did not substantially decrease shows that tRNA capable of accepting amino acids was being synthesized in the presence of CAP.

Table 1

| Treatment of Cells | O.D. 260/Liter of Culture | S. A.* CPM/O.D. 260 Leucine | Phenylalanine | Tyrosine |
|---|---|---|---|---|
| None | 94 | 39,876 | 29,096 | 8,868 |
| 1-hr CAP | 242 | 39,038 | 29,087 | 8,672 |
| 2.5-hr CAP | 318 | 38,878 | 29,887 | 7,332 |
| 4-hr CAP | 384 | 37,525 | 27,882 | 7,693 |

*The specific activities of the amino acids used were: 263, 410, and 351 μc/μmole for leucine, phenylalanine and tyrosine, respectively. Counting was approximately 50% efficient.

Figure 1A is a typical chromatogram comparing leucyl-tRNA from normal cells with that from 4-hour CAP-treated cells. The

leucyl-tRNA from normal cells is resolved into 5 distinct peaks in this chromatographic system. However, the leucyl-tRNA profile from the CAP-treated cells is strikingly more complicated. Several peaks, distinct from the normal, can be seen. The multiplicity of isoaccepting tRNAs for leucine makes it difficult to establish a relationship between the peaks observed after CAP-treatment and those normally present.

In an effort to simplify the situation, phenylalanyl-tRNA from normal cells was compared with that from CAP-treated cells. As can be seen in Figure 1B, phenylalanyl-tRNA from normal cells chromatographs as a single, distinct peak. Several peaks, chromatographically distinct from the normal, appear after CAP-treatment (Figure 1B and 1C). The

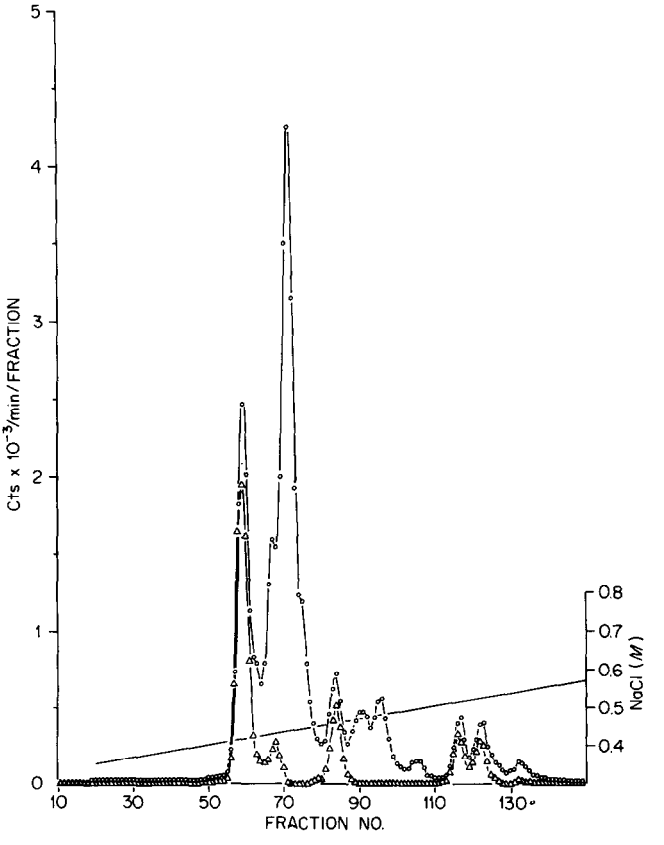


Fig. 1A

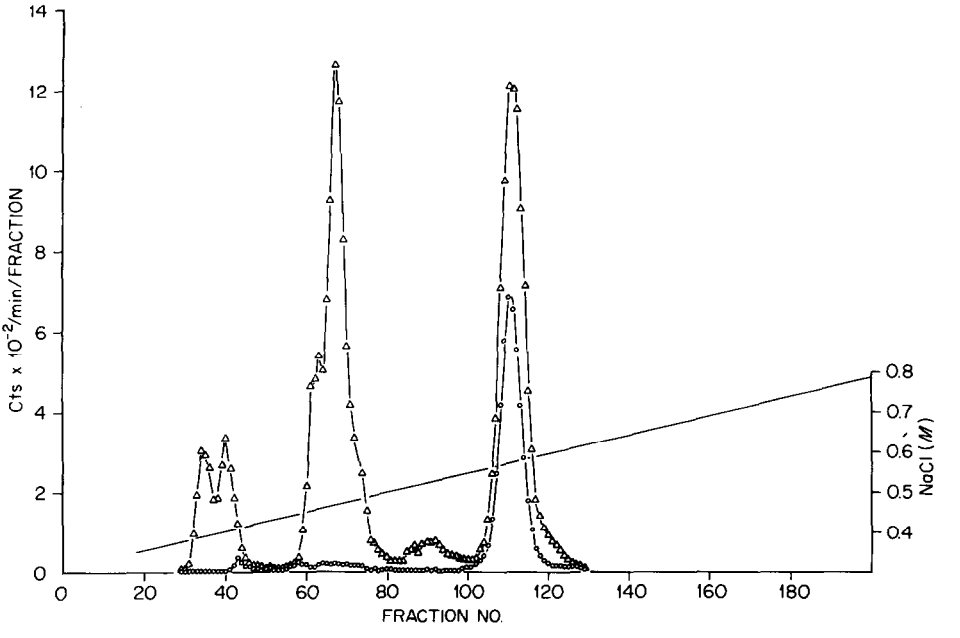


Fig. 1B

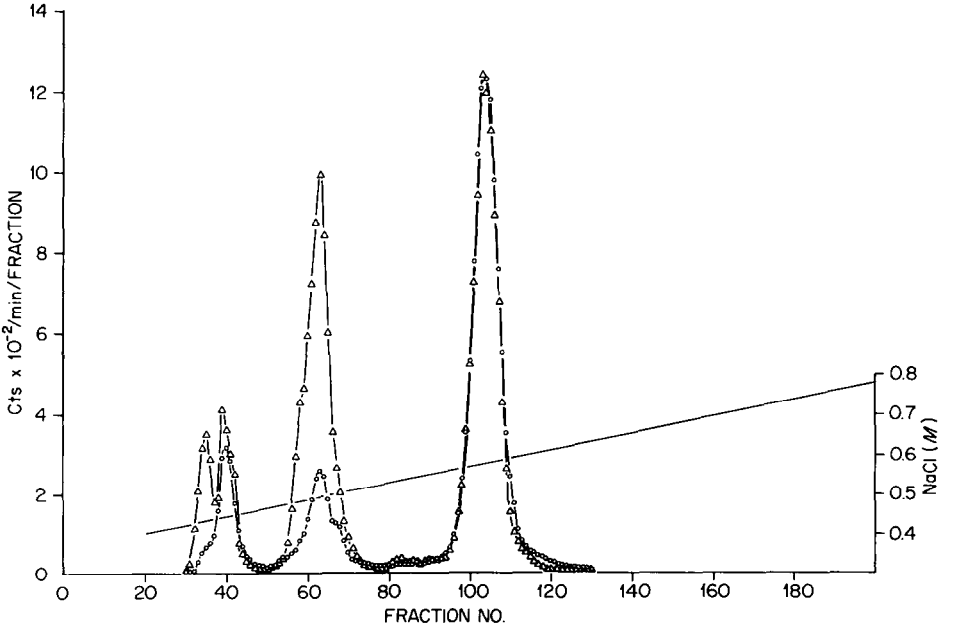


Fig. 1C

Figure 1. A. △, normal leucyl-tRNA; ○, 4-hr CAP leucyl-tRNA. B. ○, normal phenylalanyl-tRNA; △, 4-hr CAP phenylalanyl-tRNA. C. ○, 1-hr CAP phenylalanyl-tRNA; △, 2.5-hr CAP phenylalanyl-tRNA. △ = $^{3}H$; ○ = $^{14}C$

normalized data shown in Figures 1B and 1C are consistent with the following interpretations: (1) During the first hour of CAP treatment, a significant amount of phenylalanine tRNA, which elutes with the normal peak, is synthesized. However, this peak is significantly retarded. (2) Essentially all the phenylalanine tRNA synthesized subsequent to the first hour of CAP-treatment appears as new peaks. (3) The new peaks seem to be appearing somewhat sequentially, i.e., the peak eluting in fraction 40 is as prominent at 1 hour as at 4 hours, whereas the peak eluting at fraction 35 and the shoulder at fraction 58, which are prominent at 2.5 and 4 hours are essentially absent at 1 hour. An experiment in which rifampicin,* an antibiotic which inhibits RNA synthesis (Lancini et al., 1969), was added to the culture along with CAP supports the interpretation that the new peaks are newly synthesized. The phenylalanyl-tRNA isolated from the rifampicin and CAP-treated cells chromatographed as a single peak identical to that from normal cells. There was no indication of the new peaks.

An alternate interpretation is that only normal phenylalanine tRNA is synthesized after CAP-treatment and subsequent modification results in the altered chromatographic pattern. It is possible that the new peaks of aminoacyl-tRNA are degradation products derived from normal tRNA. However, when 4-hour CAP tRNA is heated and quick-cooled (Nishimura and Novelli, 1965), then charged with phenylalanine, it has chromatographic properties identical to those of an unheated sample. Chromatography on Sephadex G-100 gives no indication of degraded material in the CAP-treated tRNA samples. The experiment using rifampicin also tends to rule out degradation as an explanation for the new aminoacyl-tRNA peaks.

Because undermethylated leucyl- and phenylalanyl-tRNAs from E.

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coli had been shown to differ chromatographically from normal in the RPC systems (Waters and Novelli, 1967; Shugart et al., 1968), the possibility that undermethylated tRNA was being produced in the presence of CAP was investigated. In an in vitro system (Shugart et al., 1968), however, tRNA from CAP-treated cells did not accept methyl groups. The experiments were done with cells grown in a rich medium supplemented with methionine and cysteine. This represents an effort to rule out the possibility that one or more of the enzymes involved in the synthesis of methionine or cysteine, precursors of methyl and thiol groups, respectively, in tRNA, are labile, thus producing a precursor deficiency in CAP-treated cells. The chromatographic changes already described were still evident.

A finding, possibly analagous to that reported here, was reported recently by Gefter and Russell (1969). They showed that infection of E. coli with bacteriophage φ80, carrying a gene for a tyrosine suppressor tRNA, led to the production of 3 chromatographically separable forms of tyrosyl-tRNA. They concluded that the three forms probably differed in the relative completion of the modifications to the adenylic acid residue (normally 2-thiomethyl-$^{6}$N-isopentyl adenylic acid), found adjacent to the anticodon. It is quite probable that the multiple forms of tyrosyl-tRNA which they observed were a consequence of their use of CAP in the manipulation of the cells. We find the chromatography of tyrosyl-tRNA is altered after CAP treatment. To investigate the possibility that the tRNA, synthesized after CAP treatment, was deficient in isopentyl groups, the system described by Fittler et al., (1968) was used. It was found that neither normal nor 4-hour CAP-treated tRNA accepted mevalonic acid to any significant extent. Furthermore, the chromatography of leucyl-tRNA was not altered by permanganate treatment of tRNA from normal cells. Such treatment has been shown to enhance the isopentyl-accepting capacity of tRNA, presumably by oxidative cleavage of existing isopentyl groups (Fittler et al., 1968). Whether the chromatographic

alterations, which we observe, involve a graded series of base modifications is not certain.

Further work is required to ascertain the relationship between the new aminoacyl-tRNA peaks, unique to CAP-treated cells, and that peak or those peaks found normally. The multiplicity of new peaks for phenylalanine tRNA, which is normally characterized by a single peak, might suggest that several biochemical events are involved. This is also suggested by the sequential appearance of the new peaks. If these new peaks represent intermediates in the synthesis of tRNA, their use as substrates might serve to elucidate the biochemical pathway involved in the production of mature tRNA.

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