BIOLOGICAL EVIDENCE FOR POSSIBILITY OF THE INTRACELLULAR MECHANISM SCAVENGING 'DEFORMED tRNA MOLECULES'

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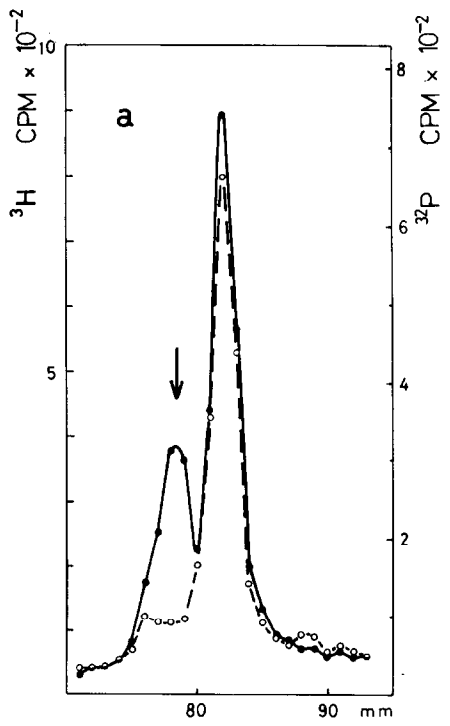
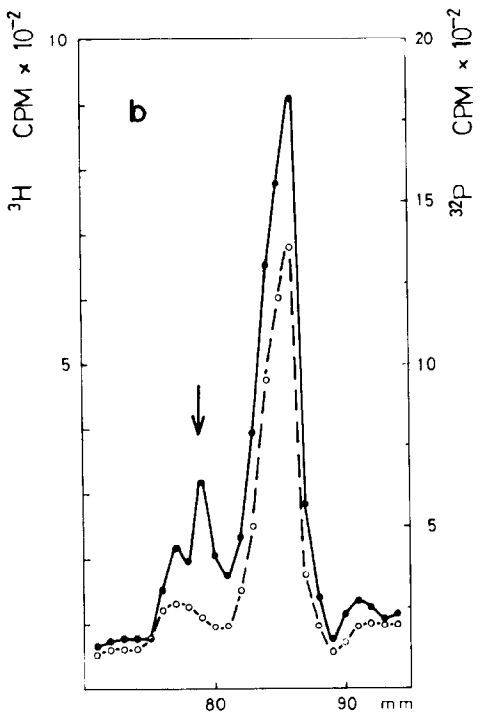
1. Introduction

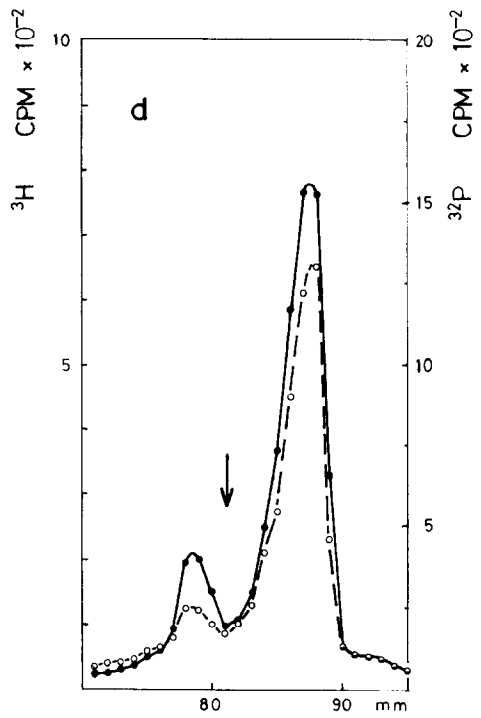
It is intended to study how organisms deal with tRNA molecules which are in a situation of structural abnormality such as conformational looseness. Previously, it was reported that in vivo suppressor activity of temperature-sensitive mutant $tRNA^{tyr}$ ($SU3^{ts\text{-}6}$ tRNA) is irreversibly inactivated at high temperature and suggested that $SU3^{ts\text{-}6}$ tRNA may be scavenged in vivo at high temperature [1]. The structure of $SU3^{ts\text{-}6}$ tRNA is not as tight as that of normal $tRNA^{tyr}$ and it is easily unfolded at relatively low temperature, but functional impairment of $Su3^{ts\text{-}6}$ tRNA appears not irreversible in vitro [2]. In order to prove the hypothesis discussed in the previous paper, experiments of polyacrylamide gel electrophoresis and DNA–RNA hybridization were performed and the selective decay of $Su3^{ts\text{-}6}$ tRNA molecules in vivo at high temperature was ascertained.

2. Materials and methods

Escherichia coli K 12, W9829 was infected with bacteriophage $\phi 80pSU3^{ts\text{-}6}$ [1], $\phi 80pSU3^{+}$ [3] or $\phi 80$ and cultured in broth containing ^{32}P at 32°C as described in the legend. One portion was shifted to 43°C and incubated for indicated periods in order to cause partial unfolding of tRNA in vivo. ^{32}P-labelled tRNA was prepared from phage infected cell as described previously [4], and fractionated by 10% polyacrylamide gel electrophoresis [5] together with *E. coli* W9829 tRNA labelled with NaB^3H_4 essentially same methods as previously reported [6]. The electrophoretic pattern was obtained as described in the legends. DNA–RNA hybridization was performed, as described in the legend, essentially the same as in previous reports [7].







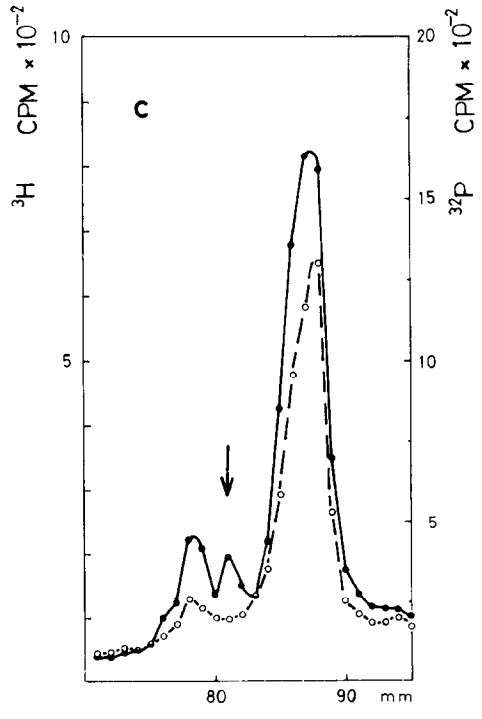


Fig. 1. Polyacrylamide gel electrophoresis of tRNA from phage infected cell. ^{32}P-labelled tRNA (specific activity was 2.6 × 10^6 cpm/nmole) from phage-infected cells was fractionated by electrophoresis on 10% polyacrylamide gel (5 mm × 130 mm, containing 40 mM Tris–acetate buffer pH 8.0, runned with 5 mA/gel) together with 3′-terminus ^{3}H-labelled control *E. coli* W9829 tRNA (specific activity was 1.6 × 10^5 cpm/nmole). Gel was frozen and cut into 1 mm slices and digested with 1 ml of H_2O_2 at 55°C for 15 hr. 15 ml of Kinard's scintillator [8] was added and radioactivity of ^{3}H and ^{32}P was counted: a) W9829 was infected with ϕ80pSU3$^+$ and cultured at 32°C in broth (1% polypeptide, 0.5% yeast extract, 0.25% NaCl and pH 7.5). Procedures of phage infection described previously [9] were modified. Cells were suspended in T1-buffer at 2 × 10^9/ml and irradiated for 22 sec with a germicidal lamp (approx. 30% survival) and infected by phage as m.o.i. of 30 for 10 min at 32°C. After dilution to 10-fold with fresh broth containing 0.07 mCi ^{32}P/ml, incubated for 25 min at 32°C and chloramphenicol added to final concn. of 50 μg/ml and cultured for 60 min at the same temperature; b) W9829 was infected with ϕ80pSU3$^{ts\text{-}6}$. All steps were performed at 32°C; c) W9829 was infected with ϕ80pSU3$^{ts\text{-}6}$. Following culture conditions 32°C for 50 min after addition of chloramphenicol, the temperature was shifted to 43°C for 10 min; d) W9829 was infected with ϕ80pSU3$^{ts\text{-}6}$. After addition of chloramphenicol, temperature was shifted to 43°C for 60 min. ——●——, ^{32}P radioactivity; ––○––, ^{3}H radioactivity.

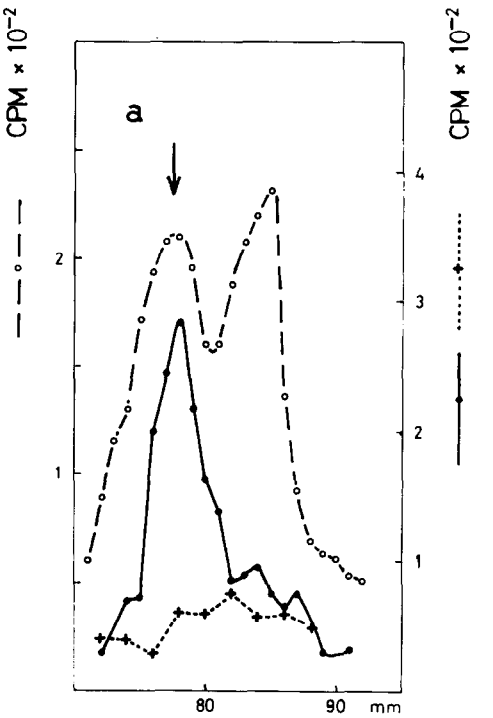
3. Results

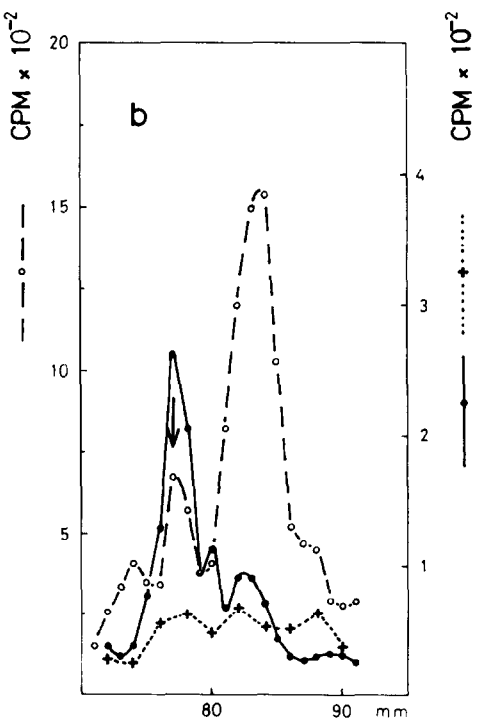
3.1. *Fractionation of tRNA from phage-infected cell by polyacrylamide gel electrophoresis*

A location of $tRNA^{tyr}$ in polyacrylamide gel electrophoresis of bulk tRNA was defined using purified *E. coli* $tRNA^{tyr\ I}$ and it is indicated with an arrow in the figures. $SU3^+$ tRNA, produced by ϕ80pSU3$^+$ infection, was greatly accumulated (fig. 1a). This pattern was not affected when the temperature of the culture was shifted to 43°C. $SU3^{ts-6}$ tRNA, produced by ϕ80pSU3^{ts-6} infection, was accumulated inefficiently even at 32° C (fig. 1b); about 10–25% of the amount of $SU3^+$ tRNA on the average, although each phage was infected to cell as same m.o.i. The amount of $SU3^{ts-6}$ tRNA accumulated in vivo declined to less than 50% after temperature shift to 43°C for 10 min (fig. 1c) and moreover resulted in an undetectable amount after incubation at 43°C for 60 min (fig. 1d). ϕ80 Wild infection resulted in the same pattern as in fig. 1d at each temperature. In vitro incubation of bulk ^{32}P-labelled tRNA from ϕ80pSU3^{ts-6}-infected cell at 43°C for 20 min in 10 mM Tris–HCl pH 7.5 and 5 mM $MgCl_2$ did not affect the position of $SU3^{ts-6}$ tRNA in the pattern of gel electrophoresis.

3.2. *Experiment of hybridization exhibiting in vivo decay of $SU3^{ts-6}$ tRNA molecules*

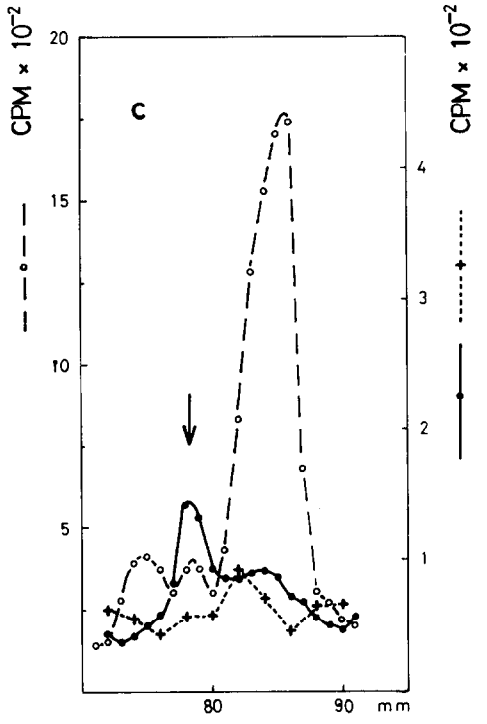
Hybridizable ^{32}P-labelled materials were detected at the same position as $tRNA^{tyr\ I}$ in the pattern of electrophoresis (fig. 2). Hybridization was inhibited by addition of cold *E. coli* $tRNA^{tyr\ I}$ to the reaction mixture. The amount of hybridizable ^{32}P-labelled materials accumulated in ϕ80pSU3^{ts-6}-infected cells declined when the temperature of the culture was shifted to 43°C for 10 min and was undetectable after incubation at 43°C for 60 min (fig. 2c and d).





4. Discussion

Biological evidence was obtained that there may be an intracellular mechanism scavenging 'Deformed tRNA Molecules' in *E. coli*. Inefficient accumulation of mutant tRNA in vivo was already reported and abnormal processing of maturation of a mutant precursor tRNA molecule was proposed as a cause of



this phenomenon [12]. In this paper, in vivo selective decay of matured mutant tRNA in the condition of making its conformation loose was reported. Therefore, it may be possible that every mutant tRNA [9,11, 12] is unstable to some extent even in the matured form and this may be one of the reasons, in addition to abnormal processing of maturation, for the low level of accumulation of mutant tRNAs. Previously, I have reported rapid and irreversible decay of in vivo suppressor activity of $SU3^{ts\text{-}6}$ tRNA at high temperature [1]. The hypothesis described in the previous paper was biochemically confirmed in this study. At present, it is clear that T4 infection is not responsible for the decay of suppressor acitivity reported in the previous paper.

In the case of protein, evidence has been presented that there are mechanisms for selective degradation of abnormal proteins [13–17] and the relation of these mechanisms to aging was discussed [18]. Similar mechanisms dealing with stable RNA would also be

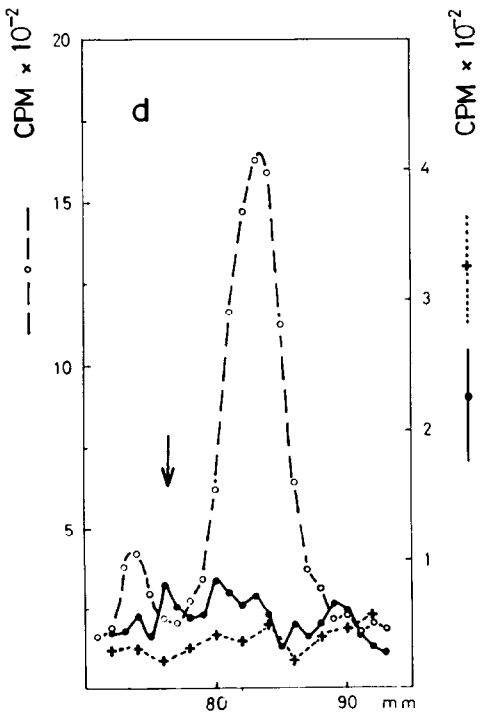


Fig. 2. Hybridization between ϕ80pSU3$^+$ DNA and tRNA from phage-infected cell. ^{32}P-labelled tRNA was fractionated by electrophoresis as described in fig. 1. Gel was cut into 1 mm slices and soaked in 1 ml of 2 × SSC (0.3 M NaCl, 0.03 M sodium citrate) overnight at room temperature, and ^{32}P-labelled materials were eluted with essentially same methods as those previously described [10]. The elution recovery was 97%. One portion of extract (5 μl) was applied on a paper disk and radioactivity was counted in toluene scintillator. Equal volumes of other parts (0.25 ml, in the case of (a); 0.1 ml, in the case of (b), (c) and (d)) were used to experiment on hybridization with akali-denatured ϕ80pSU3$^+$ DNA (10 μg for one fraction) fixed on a Millipore filter (HA 0.45) and to experiment on competition by *E. coli* cold $tRNA^{tyr}$ I (2 μg for one fraction). Hybridization was performed in 1 ml of 2 × SSC at 74°C for 15 hr and filters were washed with 2 × SSC and incubated at 30°C for 30 min in 2 × SSC containing RNase A (10 μg/ml, preheated at 80°C for 10 min) and remaining radioactivity was counted in toluene scintillator. ^{32}P-labelled tRNA's (specific activity was 4.8 × 10^6 cpm/nmole) used in (a), (b), (c) and (d) were prepared with same procedures as those of fig. 1 respectively. About 0.13 nmole of bulk ^{32}P-labelled tRNA was applied on electrophoresis in the case of (a) and 0.61 nmole was in the case of (b), (c) and (d). ——o——, eluted ^{32}P radioactivity; ——●——, hybridizable ^{32}P radioactivity; -----+-----, competition experiment.

necessary for organisms. Prominence of the turning over process of *E. coli* stable RNA under several physiological conditions has been reported [19–26] and chloramphenicol prevented this process [24]. But, in this case, it seems that degradation of r-RNA has preference to that of bulk tRNA. In contrast with general turning over of stable RNA, the phenomenon reported in this paper is characteristic from the viewpoint of being selective decay of 'Deformed tRNA Molecules' and being unprevented by addition of chloramphenicol. Biological interpretations of the relation between these two observations are very interesting.

Acknowledgement

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