# TRANSFER OF TYROSINE INTO RABBIT GLOBIN CHAIN FROM TWO ESCHERICHIA COLI TYROSYL-tRNA SPECIES

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SUMMARY

Both of two E. coli tyrosine transfer ribonucleic acids, $tRNA^{Tyr}_1$ and $tRNA^{Tyr}_2$, which have the same primary structure except for a slight difference in their nucleotide sequence in the S-region, transfered tyrosine into each of two globin chains in the rabbit reticulocyte cell-free hemoglobin-synthesizing system. Furthermore, the ratio in amounts of tyrosine incorporated from tyrosyl-$tRNA^{Tyr}_1$ and tyrosyl-$tRNA^{Tyr}_2$ was constant at every position of tyrosine residues of globin chains.

It has been reported that some isoaccepting tRNAs from several species reveal different mode of codon recognition both in the amino acid incorporation experiments using synthetic messengers of known base sequence and in the ribosomal binding experiments using triplet codons (1, 2). Furthermore, an amino acid residue at proper sequential position of globin chain has been shown to be transfered by one of the isoaccepting tRNAs using a reticulocyte cell-free hemoglobin-synthesizing system (3).

Interest in the investigations which dealt with the mode of action of the isoaccepting tRNA in the cell-free hemoglobin synthesis, however, has hitherto been directed to the problem of recognition of some degenerated codons for certain amino acid on globin mRNA by the isoaccepting tRNAs. In our laboratory, Sekiya et al. reported the specific recognition of glutamic acid codons on globin mRNA by yeast isoaccepting glutamic acid tRNAs (4) and further

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the presence of a novel relationship between the codon recognition of the yeast glutamic acid tRNA and its anticodon structure was demonstrated(5).

This time we attempted an investigation on the mode of action of two species of E. coli tyrosine tRNA in a reticulocyte cell-free system. It has already been reported that these $tRNA^{Tyr}_1$ and $tRNA^{Tyr}_2$ have entirely the same primary structure except for two bases in the S-region. Thus the UpC sequence in the S-region of $tRNA^{Tyr}_1$ is replaced with CpA in the same location of $tRNA^{Tyr}_2$ (6). In the ribosomal binding experiment, these two tRNAs indicated an indistinguishable coding specificity (7). So that, the research on the behavior in transfer of tyrosine by these two kinds of tRNAs in a cell-free hemoglobin-synthesizing system is interesting with respect to the relation between the minor sturctural difference in the S-region of tRNAs and their mode of participation in the translation of codons on natural messenger RNA.

This paper deals with the distributions in $\alpha$-globin chain of tyrosine residues transfered from E. coli tyrosyl-$tRNA^{Tyr}_1$ and tyrosyl-$tRNA^{Tyr}_2$ in the rabbit reticulocyte cell-free system.

## MATERIALS AND METHODS

Labelled amino acids were purchased from the New England Nuclear Co. Transfer $RNA^{Tyr}_1$, with a purity of about 80%, was obtained from E. coli strain B by the same procedure as used in preparing $tRNA^{Tyr}_2$. Transfer $RNA^{Tyr}_2$ was purified by column chromatography as reported previously (8, 9) and was at least 95% pure. These two tRNA preparations were not contaminated at all each other.

[$^{14}$C] Tyrosyl-$tRNA^{Tyr}_1$ and [$^{3}$H] tyrosyl-$tRNA^{Tyr}_2$ were prepared as described previously (9) by esterification of the corresponding tyrosine tRNA with uniformly labelled L-tyrosine-$^{14}$C (specific activity, 355 mCi/mmole) and L-tyrosine-3,5-$^{3}$H (specific activity, 35 Ci/mmole), respectively. Specific activity of L-tyrosine-3,5-$^{3}$H used in the control incorporation experiment was 16.5 Ci/mmole.

Preparation of a reticulocyte cell-free system and the tyrosine transfer reaction were carried out as reported in our previous paper (4).

## RESULTS AND DISCUSSION

In order to see if the both E. coli tyrosyl-tRNA could be utilized in the cell-free hemoglobin synthesizing system of rabbit, the standard reaction mixture given in the legend to Fig. 1 was incubated with several amounts of labelled E. coli tyrosyl-tRNA at 37°C for 45 min.

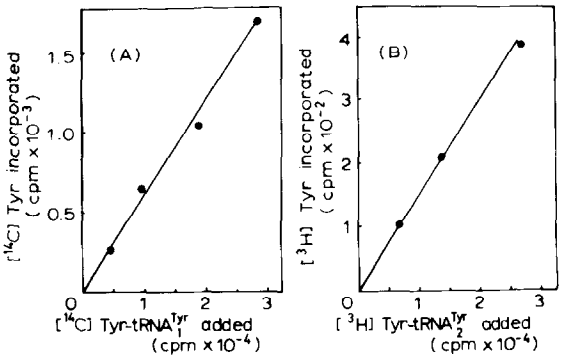


Fig. 1. Utilization of E. coli tyrosyl-tRNA for hemoglobin synthesis in the rabbit reticulocyte cell-free system. The standard reaction mixture (0.5 ml) contained 0.1 M Tris-HCl (pH 7.6), 0.025 M KCl, 3mM $MgCl_2$, 8 mM β-mercatoethanol, 0.5 mM GTP, 1 mM ATP, 0.01 M phosphoenolpyruvate, 20 μg phosphoenolpyruvate kinase, 0.2 mM nineteen kinds of unlabelled amino acids (minus tyrosine), 2 mM unlabelled tyrosine, and 50 $A_{260\ m\mu}$ units of "pH 5 pellet". A large amount of unlabelled tyrosine was added to dilute the radioactive tyrosine liberated from E. coli tyrosyl-tRNA and to minimize the formation of rabbit radioactive tyrosyl-tRNA. To the mixture appropriate amounts of radioactive tyrosyl-tRNA were added and the mixture was incubated at 37°C for 45 min. The reaction was terminated by adding 10% trichloroacetic acid solution, the mixture heated at 90°C for 10 min and the precipitate thus occurred was collected and its radioactivity was counted in Bray's solution (12) in a Packard Tri Carb Liquid Scintillation Spectometer. (A) Tyrosine transfer from [$^{14}$C] tyrosyl-$tRNA^{Tyr}_1$. (B) Tyrosine transfer from [$^3$H] tyrosyl $tRNA^{Tyr}_2$.

The Fig. 1 indicates that incorporation of tyrosine residue into the complete and incomplete globin molecule synthesized increased in linear function to the amount of the labelled tyrosyl-tRNA. The time dependent transfer of tyrosine residue into globin chains was estimated and the results are shown in Fig. 2. As is seen in the Fig. 2, the tyrosine residue was incorporated from both of the tyrosyl-tRNAs in a similar manner to the case of a control incorporation of tyrosine via endogeneous tRNA of rabbit reticulocyte in the cell-free system. Thus, in all cases, the incorporation rapidly

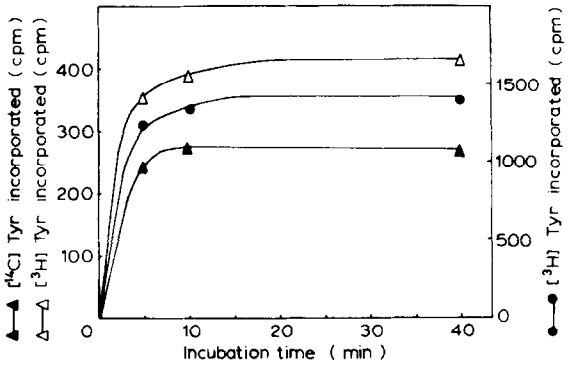


Fig. 2. Kinetics of tyrosine transfer into globin chain. The standard reaction mixture was the same as described in the legend to Fig. 1. The symbols, ▲—▲ and △—△, represent incorporation of [$^{14}$C]- and [$^{3}$H] tyrosine residues into globin chain, respectively, when the reaction mixture contained both [$^{14}$C]-tyrosyl-tRNA$^{Tyr}_{1}$ and [$^{3}$H] tyrosyl-tRNA$^{Tyr}_{2}$. The symbol, ●—●, represents incorporation of [$^{3}$H] tyrosine into globin chain via the endogeneous tRNA in a reticulocyte cell-free system, from which the addition of 2 mM tyrosine was omitted.

occurred in the first ten minutes and reached a plateau thereafter.

The incorporation of tyrosine into globin chain from E. coli tyrosyl-tRNAs was further investigated by isolation of the double-labelled hemoglobin and analyses of the radioactive tyrosyl groups in it. The reaction was performed in a standard solution (10 ml) containing 815 $A_{260\ m\mu}$ units of "pH5 pellet" (4), to which 5.35 mμmoles of [$^{14}$C] tyrosyl-tRNA$^{Tyr}_{1}$ (1.90 x $10^{6}$ cpm) and 1.53 mμmoles of [$^{3}$H] tyrosyl-tRNA$^{Tyr}_{2}$ (2.72 x $10^{6}$ cpm) were added. As a control, the transfer reaction was carried out using the standard solution (5 ml) mentioned above except for substitution of [$^{3}$H] tyrosine (40 μCi) for the labelled tyrosyl-tRNA and omission of the 2 mM unlabelled tyrosine. The labelled globin was prepared by removal of heme from hemoglobin and it was subsequently separated into α- and β-chains by carboxymethylcellulose column chromatography according to the method of Dintzis (10).

In Fig. 3a, the separation pattern of the α- and β-globin chains which were synthesized by utilizing the endogeneous tRNAs of rabbit reticulocytes is given. The Fig. 3b shows the similar pattern obtained for the double-labelled globin chains and it indicates that both [$^{14}$C]- and [$^{3}$H]tyrosines were incorpo-

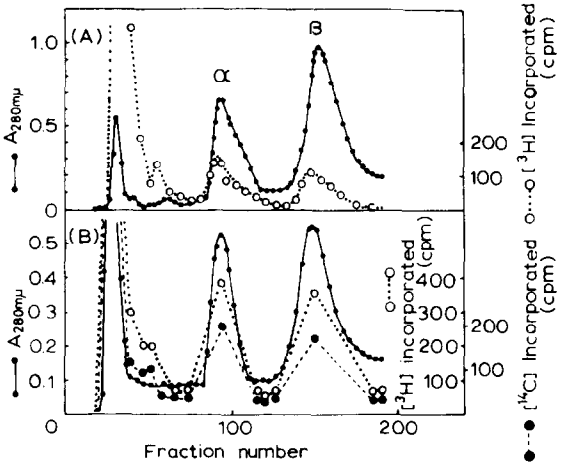


Fig. 3. Separation of $\alpha$- and $\beta$-chains of rabbit hemoglobin by carboxymethylcellulose column chromatography. The tyrosine transfer reaction was performed in a large scale as described in text. The globin labelled with radioactive tyrosine was isolated as mentioned previously (4). It was dissolved in the Solution A given below and applied to carboxymethylcellulose column (2.5 cm x 40 cm) and eluted by linear gradient solution made of 800 ml of Solution A containing 0.2 M formic acid and 0.02 M pyridine in mixing chamber and 800 ml of Solution B containing 2.0 M formic acid and 0.2 M pyridine in the reservior 7 ml fractions were collected. (A) Chromatography of globin labelled with [$^3$H] tyrosine incorporated via reticulocyte endogeneous tRNA. ●——● , $A_{280m\mu}$; o····o , radioactivity [$^3$H]. (B) Chromatography of globin doubly labelled with radioactive tyrosines incorporated from E. coli [$^{14}$C] tyrosyl-tRNA$^{Tyr}_1$ and [$^3$H] tyrosyl-tRNA$^{Tyr}_2$. ●——●, $A_{280\ m\mu}$; ●- - -●, radioactivity [$^{14}$C]; o···o , radioactivity [$^3$H].

rated into each chain of globin and that the ratio of amount of [$^3$H]- to [$^{14}$C]-tyrosine residues incorporated was almost the same for the both chains.

In order to know if there were differences between the distritubions of tyrosine transfered from tyrosyl-tRNA$^{Tyr}_1$ and tyrosyl-tRNA$^{Tyr}_2$ into the globin chains, the analysis of the radioactive tyrosine residues in the chain was carried out. It has been reported that $\alpha$-chain of rabbit globin contains tyrosine residues at positions 24, 42 and 140 from the N-terminal (11). The $\alpha$-chain obtained from the labelled globin was digested with trypsin and the tryptic peptides were separated by Dowex-50 column chromatography. The results are summarized in the Fig. 4.

The Fig. 4a shows the separation pattern of the tryptic peptides of $\alpha$-globin chain labelled with [$^3$H] tyrosine without the addition of E. coli tyrosyl-

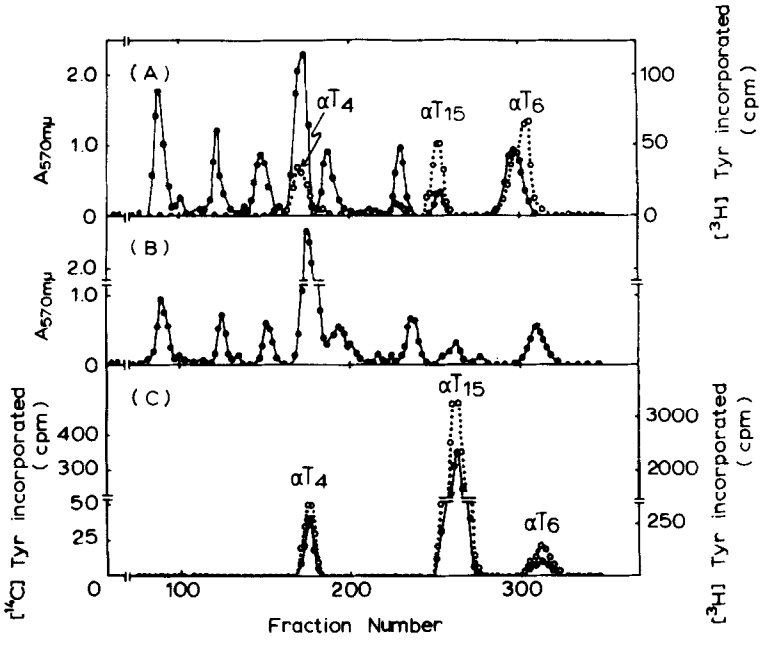


Fig. 4. Separation of tryptic peptides of the α-chain of rabbit hemoglobin by Dowex-50 column chromatography. Digestion of the α-globin chain with trypsin, which was obtained by carboxymethylcellulose column chromatography as given in Fig. 3, and Dowex-50 column chromatography of the tryptic peptide were performed according to the procedure described in our previous paper (4). 5 ml fractions of the eluate from Dowex-50 column were collected. Ninhydrin assay and determination of radioactivity were carried out using 0.2 ml and 2.0 ml aliquots of each fraction, respectively, as reported previously (4). (A) Chromatography of the tryptic peptides of the α-chain obtained by the separation of globin chain labelled with [$^3$H]tyrosine as shown in Fig. 3a. ●——●, Ninhydrin assay ($A_{570\ m\mu}$); o····o, radioactivity [$^3$H]. (B) Chromatography of the tryptic peptides of the α-chain isolated after the separation of double-labelled globin chain as shown in Fig. 3b. ●——●, Ninhydrin assay ($A_{570\ m\mu}$); (C) Incorporation of radioactive tyrosine into the fractions given in (b). ●——●, radioactivity [$^{14}$C]; o····o, radioactivity [$^3$H].

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tRNAs in a reticulocyte cell-free system. The pattern indicates that among the ninhydrin-positive peaks separated, three of them overlapped with the peaks of radioactivity. The oligopeptides contained in these peaks were isolated and identified as $\alpha T_4$, $\alpha T_6$, and $\alpha T_{15}$ peptides by amino acid analysis, which were previously reported by von Ehrenstein (11).

The Fig. 4b indicates the separation of tryptic peptides of α-chain obtained from the double-labelled hemoglobin as represented by absorbance at 570 mμ of ninhydrin coloration and Fig. 4c shows the distributions of radio-

activities in the tryptic peptides given in Fig. 4b. The Figs. 4b and 4c revealed that both the radioactivities of [$^{14}$C]- and [$^{3}$H]tyrosines localized in three peaks corresponding to $\alpha T_4$, $\alpha T_6$ and $\alpha T_{15}$ peptides and that all of these three peaks gave almost the same ratio (approx. 1:10, by cpm) in the radioactivity of $^{14}$C and of $^{3}$H.

The results indicated that every tyrosine residue in the $\alpha$-chain of rabbit globin was transfered from both of E. coli tyrosyl-tRNA$^{Tyr}_1$ and tyrosyl-tRNA$^{Tyr}_2$ in the invariable ratio and that these two kinds of tyrosyl-tRNAs were utilized indistinguishably in the translation of tyrosine codons on globin mRNA regardless of their sequential difference in the S-region.

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