SUBSTRATE SPECIFICITY OF ESCHERICHIA COLI PEPTIDYLTRANSFERASE AT THE DONOR SITE

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SUMMARY. The substrate specificity of E. coli peptidyltransferase at the donor site was investigated by the "50S reaction". Seventeen N-acetylated or unacetylated aminoacyl-tRNAs and dipeptidyl-tRNAs were used as the donor substrates and puromycin as the acceptor. Results indicated that the nature of amino acid side chain of the donor tRNA has a predominant effect on the reaction rate of peptidyltransferase. Amino acids or dipeptides with high hydrophobicity were transferred faster than those with low hydrophobicity. Amino acids with alkyl side chains are better donors than those with aromatic side chains. Substrates with C-terminal proline were transferred extremely slowly which can probably be attributed to its unusual α-imino structure in addition to its low hydrophobicity.

INTRODUCTION

Peptidyltransferase which catalyzes peptide bond formation is an integral part of the 50S ribosomal subunit (1). Convincing evidence showed that peptidyltransferase has two substrate binding sites (2). The donor site binds a peptidyl-tRNA from which the peptidyl moiety is transferred during the reaction. The acceptor site binds an aminoacyl-tRNA which accepts the peptide. The nature of the acceptor site has been studied exclusively (3-7), whereas the study of the donor site is only in its beginning (8,9).

In this study, kinetics of the transfer reaction of 17 donors, N-acetylated or unacetylated aminoacyl-tRNAs and dipeptidyl-tRNAs, were compared in the "50S reaction" (10), a modified "fragment reaction" (1). This reaction requires alcohol, cations, 50S ribosomal subunits and puromycin, whereas other components for protein synthesis are omitted. Thus it is a specific and resolved assay for peptide bond formation (1,8,10). The present paper is mainly concerned with the effect of the amino acid side chains of the donor tRNAs on the overall reaction rate of peptidyltransferase.

MATERIALS AND METHODS

Ribosomes and ribosomal subunits were prepared from E. coli B (harvested at the middle log phase) essentially according to Staehelin and Maglott (11). Cross contamination of ribosomal subunits was less than 5%. The ribosomes and its subunits were kept in liquid nitrogen until used.

The radioactive aminoacyl-tRNAs were prepared by charging E. coli tRNA with ^{14}C-labelled or ^{3}H-labelled L-amino acids with S-100 fraction. N-acetylation of aminoacyl-tRNA was done according to De Groot et al. (12). Phe-^{14}C-Phe-tRNA was prepared by condensation of N-hydroxysuccinimide ester of o-nitrophenylsulfenyl phenylalanine with ^{14}C-Phe-tRNA followed by removal of the o-nitrophenylsulfenyl group (13). N-acetyl-dipeptidyl-tRNAs were prepared by condensation of N-hydroxysuccinimide esters of N-acetyl-amino acids with various radioactive aminoacyl-tRNA (10). To identify the products, N-acetyl-aminoacyl-tRNA and -dipeptidyl-tRNA were hydrolyzed by 0.3N KOH. The cleaved N-acetyl-amino acids or -dipeptides were compared with authentic compounds by paper chromatography (n-butanol:acetic acid:water, 78:5:17) and paper electrophoresis (5% acetic acid:0.5% pyridine, pH 3.5, 17.5 volts/cm).

The "50S reaction" is similar to that of Monro's "fragment reaction" and contained (prior to methanol addition) 0.05M Tris-HCl, pH 7.5, 0.4M KCl, 0.02M $MgCl_2$, 0.05M NH_4Cl, 0.0005M puromycin, 84 μg of 50S ribosomal subunits and radioactive donor tRNA in a volume of 0.8 ml. The reaction was started by the addition of 0.4 ml of methanol and incubated at 25°. Samples of 0.1 ml were taken at timed intervals. The extent of reaction was determined by a modification (10) of the ethyl acetate extraction method of Miskin, Zamir and Elson (14). Over 90% of the puromycin derivatives was recovered by this procedure. Radioactivity was determined by liquid scintillation method. Products of the puromycin reaction were also examined by paper electrophoresis and it was found that they have electrophoretic mobility as expected (10).

RESULTS AND DISCUSSION

Kinetics of the transfer of various N-acetylated or N-unacetylated amino-

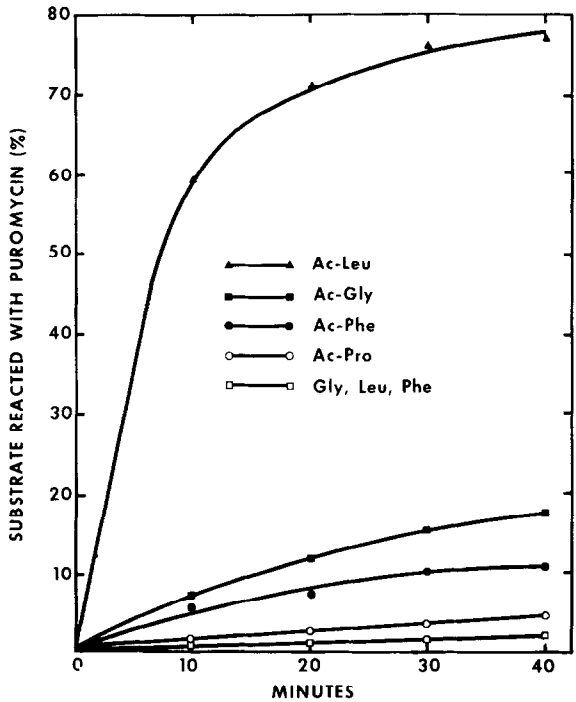

Figure 1. Kinetics of the Puromycin Reaction with N-acetylated or Unacetylated Aminoacyl-tRNA.

The reaction conditions were described under Methods. The amount of donor substrates in the reaction mixtures of 1.2 ml were either 12.8 pmoles of Ac-^{3}H-Leu-tRNA (50,430 dpm/pmole), 209 pmoles of Ac-^{14}C-Phe-tRNA (798 dpm/pmole), 912 pmoles of Ac-^{14}C-Gly-tRNA (172 dpm/pmole), 222 pmoles of Ac-^{3}H-Pro-tRNA (13,174 dpm/pmole), or equal amounts of unacetylated aminoacyl-tRNA. Samples of 0.1 ml were taken at indicated times. The extent of reaction was determined by the ethyl acetate extraction method. Each point is the mean of duplicate estimation after subtraction of the zero time blank.

acyl groups from tRNA to puromycin are shown in Figure 1. Ac-Leu-tRNA was the most active donor, about 60% of the substrate was reacted with puromycin after a 10 min. incubation at 25°. The transfer of Ac-Gly and Ac-Phe to puromycin was much slower. The most unreactive substrate among the N-acetylated substrates was Ac-Pro-tRNA. Reactivity of N-unblocked substrates such as Leu-tRNA,

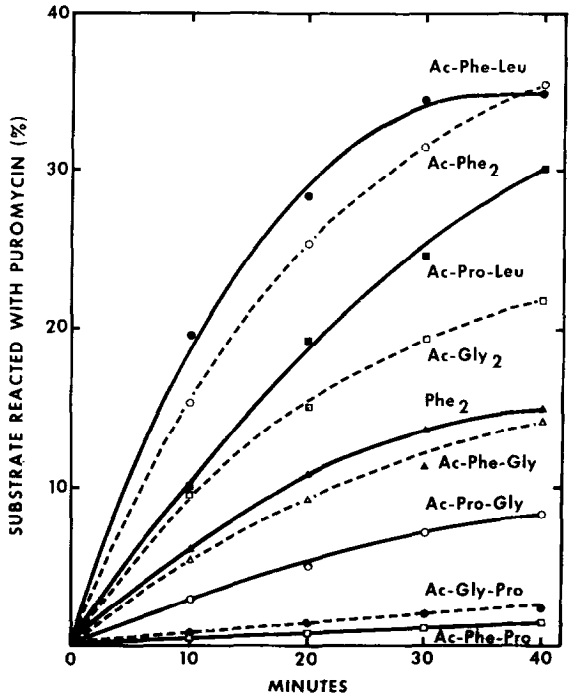

Figure 2. Kinetics of the Puromycin Reaction with N-acetylated or N-unacetylated Dipeptidyl-tRNA.

The reaction conditions were described under Methods. Reaction mixtures of 1.2 ml contained either 24 pmoles of Ac-Pro-^{3}H-Gly-tRNA (13,040 dpm/pmole), 283 pmoles of Ac-Phe-^{14}C-Pro-tRNA (445 dpm/pmole), 23.6 pmoles of Ac-Phe-^{14}C-Phe-tRNA (825 dpm/pmole), 15.4 pmoles of Ac-Pro-^{3}H-Leu-tRNA (50,430 dpm/pmole), 1472 pmoles of Ac-Phe-^{14}C-Gly-tRNA (172 dpm/pmole), 29.7 pmoles of Ac-Phe-^{3}H-Leu-tRNA (50,430 dpm/pmole), 17 pmoles of Ac-Gly-^{3}H-Pro-tRNA (13,174 dpm/pmole), 151 pmoles of Ac-Gly-^{3}H-Gly-tRNA (13,043 dpm/pmole), or 23.6 pmoles of Phe-^{14}C-Phe-tRNA (825 dpm/pmole). Samples of 0.1 ml were taken at indicated times. The extent of reaction was determined by the ethyl acetate extraction method. Each point is the mean of duplicate estimation after substraction of the zero time blank.

Gly-tRNA, and Phe-tRNA, as expected (8,9), was very low but detectable, whereas, the reaction of Pro-tRNA with puromycin was undetectable.

Figure 2 shows the reactivities of various N-acetyl-dipeptidyl-tRNA and

dipeptidyl-tRNA with puromycin. The order of the reaction rate was Ac-Phe-Leu- > Ac-Phe_2- > Ac-Pro-Leu- > Ac-Gly_2- > Phe_2- > Ac-Phe-Gly- > Ac-Pro-Gly- > Ac-Gly-Pro- > Ac-Phe-Pro-tRNA. The reaction rate of Phe_2-tRNA was slower than Ac-Phe_2-tRNA but faster than several Ac-dipeptidyl-tRNAs.

If the donor substrates are classified according to tRNA species and the donor moieties listed in the order of reactivity within the group, there are four groups of tRNA; (1) $tRNA^{Leu}$ group: Ac-Leu > Ac-Phe-Leu > Ac-Pro-Leu > Leu, (2) $tRNA^{Gly}$ group: Ac-Gly_2 > Ac-Phe-Gly > Ac-Gly > Ac-Pro-Gly > Gly, (3) $tRNA^{Phe}$ group: Ac-Phe_2 > Phe_2 > Ac-Phe > Phe, and (4) $tRNA^{Pro}$ group: Ac-Pro > Ac-Gly-Pro > Ac-Phe-Pro > Pro. Several conclusions may be drawn to shed some light on the specificity of the donor site.

Reactivity of the N-unblocked aminoacyl-tRNA is always low, which suggests that the positive charge of the α-amino group suppresses peptidyltransferase. The suppression from the charged α-amino group of the dipeptidyl moiety is less pronounced, this may be explained by the increase of distance between the charge group and the catalytic site of peptidyltransferase.

The fact that Ac-Leu-tRNA was the better substrate than all other Ac-dipeptidyl-tRNAs suggested that the number of amino acid residue on tRNA is not a decisive factor on the rate of peptidyltransferase. A similar conclusion can be drawn from $tRNA^{Pro}$ series in which Ac-Pro-tRNA was a better substrate than Ac-Gly-Pro-tRNA and Ac-Phe-Pro-tRNA.

It appears that the nature of the amino acid side chain, particularly the hydrophobicity, is the most important factor determining the reaction rate of peptidyltransferase. The hydrophobicity of amino acids is in the order of Leu > Phe > Gly > Pro, and hydrophobicity of dipeptides is expected to be additive of the two amino acids. Aminoacyl-tRNAs and dipeptidyl-tRNAs which contained amino acids with high hydrophobicity were better substrates for peptidyltransferase than those which contained amino acids with low hydrophobicity. Actually the hydrophobicity of Phe is quite close to that of Leu, yet the reactivity of Phe-containing substrates were much lower than Leu-containing sub-

strates. This may indicate that the aromatic side chain is less favorable than alkyl side chains for peptidyltransferase.

This hypothesis that hydrophobicity of amino acids of the donor tRNA dictate the reaction rate of peptidyltransferase is not only applicable to substrates with the same tRNA but also applicable to substrates with different tRNAs. This suggests that the structural differences of various tRNAs play a less important role than amino acid.

The low reactivity of substrates with Pro at N-terminal, i.e. Ac-Pro-Leu-tRNA and Ac-Pro-Gly-tRNA, can be traced to the low hydrophobicity of Pro. However, the particularly low reactivity of substrates with C-terminal Pro, (Ac-Gly-Pro-tRNA and Ac-Phe-Pro-tRNA) probably is not only due to low hydrophobicity but also due to the peculiar α-imino group of Pro. It is plausible assuming that peptidyltransferase is designed to transfer a donor with α-amino group rather than α-imino group.

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