BIOORGANIC CHEMISTRY 2, 286–292 (1973)

A Detailed Model of the Active Centre of *Escherichia coli* Peptidyl Transferase[1]

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Received September 1, 1972

On the basis of published data, a detailed model of the active centre of *Escherichia coli* peptidyl transferase is proposed. The major conclusions are as follows: A binding site is present at each of the acceptor (A′) and donor (P′) substrate binding sites of the enzyme for the 3′-terminal CpCpA of aminoacyl- and peptidyl-tRNA, respectively. In particular, the acceptor CpCpA binding site is composed of sites for the following groups: the terminal adenine, the first phosphoryl residue from the 3′-terminus, the 3′-penultimate cytosine, and the second 3′-CMP residue. In addition, two binding sites are present on each of the A′ and P′ sites, one for the basic and one for the hydrophobic aminoacyl R groups of both aminoacyl-tRNA and the carboxyl-terminal amino acid of peptidyl-tRNA. The role of these sites in the binding of inhibitors and substrates and in the mechanism of catalysis of peptide bond formation by peptidyl transferase is discussed.

INTRODUCTION

Peptidyl transferase catalyses peptide bond formation during protein synthesis on the ribosome by a process involving the transfer of a nascent peptide chain from peptidyl-tRNA in the P site to aminoacyl-tRNA in the A site (*1*). The transfer may involve a nucleophilic attack of the α-amino group of aminoacyl-tRNA on the carboxyl ester link of peptidyl-tRNA with the subsequent cleavage of the ester and formation of a peptide bond (*2–4*). However, relatively little is known about the precise mechanism of catalysis or the nature of the active site. We therefore propose a model of the active centre of *E. coli* peptidyl transferase which is an extension of a theory on the mechanism of action of a number of inhibitors and substrates of this enzyme (*5*). It is an attempt to explain and correlate some of the structure–activity relationships of these and other compounds which act on peptidyl transferase.

[1] *Abbreviations:* A-L-amino acid, 2′(3′)-*O*-L-aminoacyl adenosine; Ac-L-phe-CH_2Cl, *N*-acetyl-L-phenylalanyl chloromethylketone; CpA-F-met, cytidylyl-(3′-5′)-2′(3′)-*O*-(*N*-formyl-L-methionyl) adenosine (other *N*-blocked and unblocked 2′(3′)-*O*-aminoacyl oligonucleotides are similarly abbreviated); C-L-phe, G-L-phe, I-L-phe and U-L-phe, the 2′(3′)-*O*-L-phenylalanyl derivatives of cytidine, guanosine, inosine and uridine, respectively; TLCK, α-*N*-tosyl-L-lysyl chloromethylketone; TPCK, α-*N*-tosyl-L-phenylalanyl chloromethylketone; Z-gly-L-phe-NA, benzyloxycarbonyl-glycyl-L-phenylalanyl nitroanilide, Z-L-phe-ONP, benzyloxycarbonyl-L-phenylalanyl-*p*-nitrophenyl ester.

MODEL OF THE ACTIVE CENTRE OF PEPTIDYL TRANSFERASE

The acceptor or A site and the donor or P site of the 70S bacterial ribosome responsible for the binding of aminoacyl- and peptidyl-tRNA, respectively, are shown diagrammatically in Fig. 1 (*6*). The A′ and P′ sites are sections of peptidyl transferase responsible for the binding of the 3′-terminal parts of these molecules and are involved in peptide bond formation (*1*, *7*). The model outlined below is an attempt to define the nature of the A′ and P′ sites and to outline aspects of the binding to these sites of the various antibiotics and substrates including those considered in Ref. 5. In particular Fig. 2 of Ref. 5 should be consulted for structural comparison of the various A′ site specific compounds.

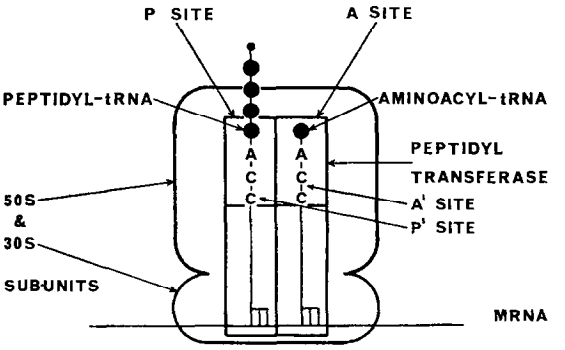

FIG. 1. Diagrammatic model of *E. coli* ribosome showing A and P sites occupied by aminoacyl- and peptidyl-tRNA and the A′ and P′ sites of peptidyl transferase occupied by the 3′-terminal portions, CpCpA-amino acid, and CpCpA-peptide, respectively.

1. A′-Site of Peptidyl Transferase

The A′ site of peptidyl transferase is responsible for binding the 3′-terminal end of aminoacyl-tRNA during peptide bond formation. Specifically, the substrate binding sites given below are proposed to form the A′ site (Fig. 2). In addition, details of the binding of the following A′ site specific compounds to the various substrate binding sites are considered; puromycin and various analogs, I-L-Phe, C-L-Phe, chloramphenicol, Ac-L-Phe-CH_2Cl, TPCK, Z-L-Phe-ONP, Z-Gly-L-Phe-NA, CpCpA-amino acid, celesticetin, lincomycin, sparsomycin, blasticidin S, gougerotin, amicetin, 3′-*N*-homocitrullyl 3′-aminoadenosine, 3′-*N*-lysyl 3′-aminoadenosine, streptothricin, and TLCK (*5*).

a. A hydrophobic site, I, which is relatively specific for the binding of the aromatic aminoacyl R groups of A-L-Phe, A-L-tyr and of the L-phe, S-benzyl-L-cys and *O*-benzyl-L-ser aminoacyl analogs of puromycin. The less hydrophobic 2′(3′)-*O*-aminoacyl adenosine and 3′-*N*-aminoacyl-puromycin aminonucleoside derivatives of L-met, L-leu, L-val, L-ala, L-pro, and gly, are bound with lower affinity (*8*, and references therein). The high acceptor activity of the more hydrophobic derivatives can be understood in terms of binding to this site. Similar hydrophobic interactions have been inferred by Nathans and Neidle (*9*) and Rychlik et al. (*10*).

This site is also implicated in the binding of the *p*-methoxy-benzyl group of puromycin, the benzyl group of I-L-phe and C-L-phe, the nitrophenyl group of chloramphenicol, (model 3 in Fig. 2 of Ref. 5), the benzyl group of the L-phe residue of *N*-Ac-L-phe-CH_2Cl, TPCK, Z-L-phe-ONP and Z-gly-L-phe-NA and the 4′pentyl group of

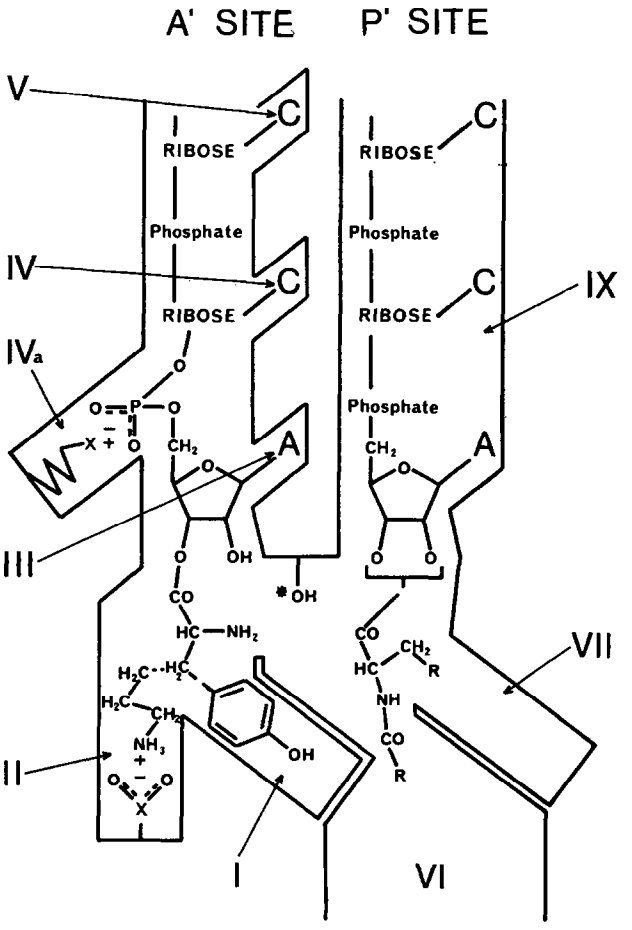

FIG. 2. Diagrammatic model of the active centre of *E. coli* peptidyl transferase. Details of the sites I–IX (VIII not shown) are given in the text. Attachment is shown to peptidyl transferase of the CpCpA-amino acid (A′ site) and CpCpA-peptide (P′ site), the 3′-termini of aminoacyl- and peptidyl-tRNA, respectively.

The sites I and II are shown occupied by the R groups of L-tyr and L-lys. Based on the activity of the 2′- and 3′-isomers of puromycin (9), the 3′-isomer of CpCpA-amino acid is depicted as the active isomer. Peptide bond formation may proceed via direct attack of the α-amino group of aminoacyl-tRNA on the carboxyl-terminal ester link of peptidyl-tRNA. Alternatively, the possibility of an enzyme-peptide intermediate (via a serine* residue?) is indicated.

lincomycin (*5*). The increased *in vivo* activity of lincomycin analogs in which the length of the 4′-pentyl chain is increased (*11*) is consistent with this binding to the hydrophobic site. The site presumably functions *in vivo* to bind the aminoacyl R groups of the various hydrophobic aminoacyl-tRNA molecules.

b. A hydrophilic site, II, which is involved in binding, via a coulombic attraction between a negatively charged group on the ribosome (e.g., an α-, β- or γ-carboxyl or a phosphoryl group) of the following positively charged groups: the methylguanidinium group of blasticidin S and the ε-amino group of streptothricin, 2′(3′)-*O*-L-lysyl adenosine and TLCK (*5*). The uncharged 6-methyluracil group of sparsomycin and the ureido group of 3′-*N*-homocitrullyl 3′-aminoadenosine may also bind via hydrogen bonding to this site (*5*). Rychlik et al. (*10*) have recently proposed a similar site based on the high acceptor substrate activity of A-L-lys for *E. coli* peptidyl transferase activity as compared to the negligible activity of A-gly. The site presumably functions similarly *in vivo* to bind the aminoacyl R groups of arg- and lys-tRNA.

c. A site, III, for the binding of the 3′-terminal adenine of aminoacyl-tRNA, the dimethyladenine of puromycin, the hypoxanthine of *I*-L-phe, the cytosine of C-L-phe, blasticidin S, gougerotin and amicetin, and the heterocyclic group of streptothricin. The heterocyclic groups of the inactive acceptor substrates, G-L-phe and U-L-phe (*12*) are presumably not bound to this site.

d. A site, IV, specific for the binding of the 3′-penultimate residue (3′-CMP) of aminoacyl-tRNA, previously proposed by Harris et al. (*8*). This site may also be involved in the binding of the salicylyl group of celesticetin.

e. A site, IVa, for the binding, via a coulombic attraction to a positively charged lysine or arginine R group on the ribosome, the first phosphoryl group from the 3′-end of aminoacyl-tRNA and the 5′-carboxyl group of blasticidin S. The greater acceptor activity of 2′(3′)*O*-L-phenylalanyl-(5′-*O*-methylphosphoryl adenosine) as compared to A-L-phe (*13*) can be explained in terms of binding to this site. In addition, the 5′-amide group of gougerotin may bind by hydrogen bonding to this site; consistent with this is the manyfold reduction in inhibitory activity of a gougerotin analog lacking the 5′-amide group (*14*).

f. A site, V, specific for the third nucleotide (3′-CMP) from the 3′-terminus of aminoacyl-tRNA, as previously pointed out from the data of Takanami (*15*) and Scolnick et al. (*16*) by Harris et al. (*8*).

2. P′ Site of Peptidyl Transferase

This site is responsible for binding the 3′-terminal end of peptidyl-tRNA during peptide bond formation. Details of the substrate specificity of the P′ site are limited to those of Monro et al. (*17*) on the activity of the various α-*N*-acyl-aminoacyl-oligonucleotides as donors in the fragment reaction (*18*), in which a blocked α-amino group is essential for high activity as compared to the unblocked derivative (*17*).

Specifically, the following substrate binding sites are proposed to form the P′ site (Fig. 2): A hydrophobic site, VII, for the binding of the aminoacyl R groups of the hydrophobic amino acids, and a hydrophilic site, VIII (not shown), for binding the basic guanidinium group of the arginyl residue are proposed in view of the high donor activity of α-*N*-acyl-aminoacyl-oligonucleotide derivatives of L-met, L-phe, L-leu, and L-arg, compared to the very low activity of α-*N*-acetyl-glycyl-oligonucleotide (*17*). On the basis of the inactivity of CpA-F-met and CpA-Ac-leu compared to the high donor activity of CpCpA-F-met and CpCpA-Ac-leu (*17, 19*), a site (shown as IX in Fig. 2) is

proposed (see also Monro et al., *17*) for the binding of the CpCpA portion of the various α-*N*-acyl-aminoacyl-oligonucleotides.

In addition to the above A′ and P′ sites, a site VI for the newly synthesized peptide is proposed in view of the known protection of nascent peptides from proteases (*20*). This site is possibly occupied by the *N*-methylglycyl residue of gougerotin, the dichloroacetyl group of chloramphenicol (model 3 in Fig. 2 of Ref. 5) and the *N*-aminoacyl residues of the *N*-aminoacyl analogs of chloramphenicol, the tosyl groups of TLCK and TPCK, the *N*-acetyl group of *N*-Ac-L-phe-CH_2Cl, the Z-gly of Z-gly-L-phe-NA, the Z-group of Z-L-phe-ONP, and the polylysine side chains of the streptothricins (*21*). In the case of these compounds, therefore, part of each of the respective molecules also binds to site VI, the site for the nascent peptide, in addition to binding to the A′ site.

3. Mammalian Peptidyl Transferase

Details of mammalian peptidyl transferase are scant, but of considerable importance in the understanding of the bacterial specificity of chloramphenicol, celesticetin and lincomycin, and essential for the rational design of selective antibacterial compounds. However, the activity on mammalian peptidyl transferase of puromycin and various analogs (*8, 22*), sparsomycin, blasticidin S, gougerotin, and amicetin (*23–25*) indicates that sites I–IV may form part of the mammalian enzyme.

DISCUSSION

The above conclusions rely on data on the substrate specificity of peptidyl transferase which have not distinguished between affinity of the substrates for peptidyl transferase and the rate of reaction once the substrates were bound (*17*). Recent studies on the affinity of various 2′(3′)-*O*-aminoacyl-oligonucleotides, produced by T1 ribonuclease digestion of aminoacyl-tRNA, for the A′ site of *E. coli* peptidyl transferase, have shown that the phe-oligonucleotide had greater affinity than the ala, glu, leu, met, ser, or val oligonucleotides (*26, 27*). These data are consistent with the existence of site I. However, the composition of the various oligonucleotides distal to the CpCpA terminal sequence could influence the biological activity of the various α-*N*-acetylated and unsubstituted 2′(3′)-*O*-L-aminoacyl-oligonucleotides, but this possibility is not supported by the small differences in extent of binding to the A′ site of CpApCpCpA-phe versus CpCpA-phe or

CpApCpCpA-leu
or
UpApCpCpA-leu

versus CpCpA-leu (*27*). Further, oligonucleotide-F-met fragments in the range CpCpA-F-met to CpApApCpCpA-F-met gave approximately equivalent activity as donor substrates (*17*). Data on the affinity of *N*-blocked aminoacyl-oligonucleotides are limited as only the binding of CpApCpCpA-Ac-leu has been studied (*28*).

The *in vivo* role of the binding sites proposed in Fig. 2 may be as follows: collectively sites III, IV, IVa, and V at the A′ site and IX at the P′ site may act as recognition sites for peptidyl transferase for the CpCpA termini of aminoacyl- and peptidyl-tRNA (*17*),

while sites I and II of the A′ site may restrict movement of the α-amino group of aminoacyl-tRNA and orientate it in a favourable configuration for an attack on the carboxyl ester link of peptidyl-tRNA. These two latter sites may also function to prevent interference from the various aminoacyl R groups with the transferase reaction. Similar proposals are forwarded for the function of the binding sites VII and VIII of the P′ site (*8*, *13*).

From the data and conclusions considered here, the A′ and P′ sites of peptidyl transferase show similarities with respect to substrate specificity; both have binding sites specific for the CpCpA termini of aminoacyl- and peptidyl-tRNA and binding sites for the basic and hydrophobic aminoacyl R groups of aminoacyl- and peptidyl-tRNA. However, the P′ site is more restrictive in that α-*N*-acyl-aminoacyl-oligonucleotides require the minimal sequence CpCpA for activity as donor substrates, while at the A′ site various aminoacyl adenosine derivatives act as acceptor substrates. The essential requirement for the CpCpA portion on the P′ site may explain why many antibiotics affect only the A′ site (*5*). With regard to specificity at the A′ site and the role of peptidyl transferase in polypeptide termination (*29–31*), it appears that even water acts as an acceptor substrate. However, the mechanism by which the normal specific requirements for acceptor activity are waived under the direction of various terminating codonds and factors is not known.

Models for peptidyl transferase involving 5S ribosomal RNA have been proposed (*32*, *33*) but there is no direct evidence to implicate 5S RNA in the action of this enzyme. In fact ribosomes lacking 5S RNA, or containing chemically modified 5S RNA, have significant peptidyl transferase activity (*34*, *35*).

In view of the above considerations it appears that peptidyl transferase is a complex enzyme possibly consisting of two or more subunits. We hope that this model will stimulate further investigations. Certainly development of selective chemotherapeutic compounds may be possible when molecular details of bacterial and mammalian peptidyl transferases are known.

ACKNOWLEDGMENT

This work received financial support from the Australian Research Grants Committee.

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