Peptidyl transferase centre of bacterial ribosomes: substrate specificity and binding sites.

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SUMMARY

A detailed scheme of the Peptidyl Transferase Centre of bacterial ribosomes is proposed by summarizing the literature data on the substrate specificity of the acceptor and donor sites. According to the proposed scheme only the elements of the donor and acceptor having a stable structure bind with the ribosome. The present paper proposes such main elements for the donor and acceptor.

The Peptidyl Transferase Centre (PTC) which performs the catalysis of the peptide bond formation, is localized on the large ribosomal subunit (50S for the bacterial ones)^{1,2}. Its immediate surrounding is not completely revealed, however it is known that a number of proteins and the 23S rRNA take part in its formation. Fig. 1³ shows the general view of the PTC structural organization in the plane. Peptide bond formation does not require additional sources of energy. The necessary energy is already present in the structure of aminoacyl-tRNA (and peptidyl-tRNA respectively) and is released in the process of the peptide residue transfer from peptidyl-tRNA to the NH₂-group of aminoacyl-tRNA⁴.

Thus the PTC-catalyzed reaction is a two-substrate one. However in comparison with simple two-substrate systems it has this peculiarity that in the process of peptide chain formation, the structure of these substrates is different in certain parts. In the acceptor (A-site) of the PTC, the complete set of aminoacyl-tRNA existing in the cell is bound alternatively (this set consists of about 80 aminoacyl-tRNA's) and the donor (D-site) receives the same set in

Fig. 1. Structural organization of the 50S subunit of the E.coli ribosome near the PTC (according to ³).

the process of translocation but the amino acid residue is incorporated in the peptide whose structure can practically have an infinite number of variants. Therefore it can be expected that the A-site as well as the D-site of the PTC must have the elements of recognition of the specific substrate components and the elements allowing them a great freedom in choosing the substrate (Fig. 2).

Fig. 2. Constant (1) and variable (2) parts of substrates of aminoacyl-tRNA and peptidyl-tRNA.

An intensive study of the PTC substrate specificity is being carried out using model PTC substrates capable of being donors or acceptors of the peptide in the one-stage process of the peptide bond formation. Studies of this kind began in 1962-1965 with the determination of the working mechanism of the antibiotic puromycin which is a model acceptor⁵⁻⁷ and with the demonstration in 1966 of the possibility of using the amino acid nucleoside esters for the same purpose⁸. In 1967 terminal peptidyl-tRNA fragments containing the minimum three nucleotide CCA sequence were applied to the D-site⁹; in 1973 after a modification of the system it was proved that certain peptide esters of the adenylic acid can also be peptide donors^{10,11}.

As it is known, all the functionally active tRNA's have the similar trinucleotide CCA at the 3'-end, but the remainder of the nucleotide tRNA sequence is different for each type. Numerous experiments have shown that the CCA-aminoacyl binds actively with the A-site of the PTC¹² while the CCA-acylaminoacyl (modeling the CCA-peptidyl) binds to the D-site^{9,13}. Therefore it is possible that the CCA fragment is recognized by both PTC sites. The first hypothetical scheme of A and D-sites of the PTC was formulated on this basis¹⁴. However the appearance of a great number of studies during the last 2-4 years with model substrates demands their generalization and an attempt to deepen as well as to revise partly the suggested hypothesis.

The formulation of requirements, minimal but sufficient for displaying the activity, to the substrates of the A and D-sites permits the expression of a definite idea about PTC properties and the choice of a convenient model for the PTC conformational study.

PTC ACCEPTOR SITE.

The investigation of the substrate specificity of the A-site of the PTC was carried out using amino acid esters or amides of nucleosides or their derivatives. Table 1 lists the literature data on the PAA of the best studied peptide model acceptors. All the compounds in this table are divided into three groups: substances with a high PAA; medium (or varying depending on the system of investigations); and substances practically devoid of the PAA. A systematic analysis of specificity of the A-site of the ribosome to different elements of model acceptor is given below.

Base residue in the nucleotide component. Derivatives with adenine, hypoxanthine and cytosine, but not with uracil and guanine, possess the PAA (Table 1). The first conclusion that can be drawn from these data is that specificity of the A-site

Table 1: Substrate Specificity of Model Peptide Acceptors

Highly active peptide acceptors	Peptide acceptors with a comparatively small activity	Substances which practically do not accept the peptide
A-Phe, I-Phe ^{15,16}	C-Phe ^{15,16}	U-Phe, G-Phe ^{15,16}
A-Phe, A- ^{OMe} -Phe ^{17,18}		A-H ¹⁶ , A-Phe ²⁰
A-OH-Phe (or) ¹⁹		A-Phe ²⁰
A-Phe, A-OH-NH-Phe ²¹⁻²³		A-NH-Phe ²¹ , Ade-Gluc-Phe
Ade-HACP-Phe ²⁴⁻²⁶		Ade-Ara-NH-Phe ²⁷
CpA-Phe ²⁰ , CpA-OH-NH-Phe ²⁰	CpA-Gly ²⁹	U* ^p A-Phe, A* ^p A-Phe ³⁰
C* ^p A-Phe ^{21-23,28}	CpA-OH ^{21-23,28}	
	CpA-NH-Gly	
CpA-Phe ^{20,31}		C ^p A-Phe ³¹ , CpA- ^{OMe} -Phe
		CpA-Phe ³²⁻³⁴
		CpA-H
A-Phe, A-Tyr ^{15,16} 21-23,28,35,36	A-Lys, A-Met, A-Ala, A-Leu, A-Val, A-Ser, A-Pro and corresponding amides ^{15,16,21-23,28,35-36}	A-Gly, A-Trp, A-OH-NH-Gly ^{15,16,21-23,28,35,36}
pA-Phe, MepA-Phe ³⁷	pA-Val, pA-Pro ³⁷	pA-Gly, pA-D-Phe ³⁷
A-L-Phe ^{15,16}		A-D-Phe ^{15,16}

is not absolute even in the range of the main types of nucleosides. The second conclusion is that among three compounds active as acceptors (A-Phe, I-Phe and C-Phe) we cannot reveal any similar structural elements in the bases (NH₂ or OH-groups, cyclic system dimensions, etc.) except that they are always aromatic structures.

Ribose residue. The ribose 2'-hydroxyl in A-Phe can be methylated almost without the loss of activity by A-^{OMe}-Phe. At the same time its complete removal leads to the loss of the PAA (by A-H⁻-Phe). However the absence of the 2'-hydroxyl in the 3'-terminal adenosine in the dinucleoside phosphate of CpA-H⁻-Phe does not influence the PAA. Thus the 2'-hydroxyl does not play an essential role in binding with the A-site. Endocyclic furanoside oxygen can be substituted by the CH₂-group almost without the loss of the PAA (Ade-HACP-Phe). Aminoacyloxy- as well as aminoacylamido-groups can be in the 3'-position, the PAA of such esters or amides is practically unchanged (A-Phe and A-OH-NH-Phe, A-Tyr and A-OH-NHTyr, etc.).

The hydroxymethyl group is not obligatory either and can be substituted by hydrogen (Ade-HACP-Phe). The intactness of the ribose ring is not necessary for PAA, thus A^{-OH}_{-Phe} (or) in which the bond between C₂' and C₃' is disrupted, accepts the peptide well.

At the same time the ribose residue cannot be replaced by arabinose or glucose:

Ade-Ara-NHPhe and Ade-Gluc-Phe do not possess PAA. Also, the amino acid residue must be in the 3'-position and not in the 2' one (A^{-NHPhe}_{-OH}, A^{-Phe}_{-OMe}, CpA^{-Phe}_{-H} as peptide acceptors are not active). It is obvious from the given data that there is no element of the ribose structure in the "minimal" acceptors necessary for the maintenance of PAA but it is important that it should be D-ribose and that the amino acid should be in the 3' and not in the 2'-position.

Substituent in the 5'-position. The PAA somewhat increases in the series A-Phe, pA-Phe, MepA-Phe though this increase is not large by its absolute value. CpA^{-OH}_{-NHPhe}, CpA-Phe and C^ppA-Phe possess a high PAA; C^pA-Phe, U^ppA-Phe and A^ppA-Phe do not accept the peptide. In the range of glycine esters CpA-Gly and CpA-NHGly had a varying PAA (depending on the system of investigations) but UpA-NHGly, ApA^{-OH}_{-NHGly}, GpA^{-OH}_{-NHGly} 22, 23, 28 and UpA-Gly 29 did not display activity. It follows from these data that the nucleotide in the 5'-position plays a considerable role in displaying the PAA of model substrates. Only the phenylalanine and glycine esters of CpA, i.e. the analogs of the aminoacyl-tRNA derivatives, are active. The PAA of these compounds is understandable.

B = Ura U^ppA-Phe
B = Ade A^ppA-Phe
B = Cyt C^ppA-Phe

It is not clear why U^ppA-Phe, A^ppA-Phe and especially C^pA-Phe are not active. In their structure all of them contain the elements necessary for displaying a high PAA,

namely, the residue A-Phe. Consequently, the 5'-substituent in these compounds distorts in some way this necessary element thus removing the PAA. It could be presumed that only the cytidine residue can be the 5'-terminal while uridine, guanosine or adenosine are not bound by the corresponding zones of the A-site (they are possibly even repulsed). However this point of view can be criticized. First, C^pA-Phe having the cytidine residue in the 5'-position does not possess PAA. Second, it is difficult to explain why the uracil residue in U^pA-Phe is repulsed by the corresponding region of the PTC since it is similar in dimensions to cytosine. It must be remembered that even the 3'-terminal nucleoside in model acceptors does not display absolute specificity, since A-Phe, I-Phe and C-Phe are also active. A conclusion can be drawn from the above that the 5'-nucleotide structure in amino acid derivatives of dinucleoside phosphates is very important for displaying PAA.

Amino acid residue. The most obscure feature is the dependence of the PAA of amino acid esters or amides of adenosine on the nature of the amino acid residue, since in the aminoacyl-tRNA all the amino acid residues accept the peptide well. Even in the case of the dinucleoside phosphate CpA-aminoacyl both the best amino acid, phenylalanine, and the worst, glycine, are active. The attempts to explain the PAA for A-Phe and A-Tyr by the existence of a hydrophobic "pocket" and for A-Lys by a cation-binding centre made in the model by Harris and Symons¹⁴, in our opinion, are open to criticism. First, A-Trp also containing the hydrophobic aromatic structure is not active. Second, it is difficult to imagine the existence of special binding sites for variable components of the substrate. We think that it is more probable that the amino acid residue structure creates a favourable conformation of the acceptor at the expense of thermodynamic parameters.

Requirements of the A-site of the PTC to its substrate. From the analysis of the above given data it can be seen that for the PAA to be present, the amino acid ester or amide of a nucleoside must satisfy three requirements:

- (1) to have a common molecular conformation satisfying the A-site of the PTC;
- (2) to contain a hydrophobic aromatic part (base); and (3) to have an amino- (or hydroxy-) group fixed in a strictly determined way relative to the aromatic part of the molecule.

It is possible to imagine that the A-site of the PTC is located in a certain cavity

near the surface of the 50S ribosomal subunit. This cavity has a definite shape and thus can bind the substrate complementary to this form. Introduction of the rule concerning the requirement of the A-site to the general conformation of the molecule permits one to explain all the contradictions. It is known that the conformation of dinucleoside phosphates is determined by the nature of the constituent nucleosides and differs for each pair³⁸. A*_pA-Phe and U*_pA-Phe are not active because the conformation of the residue-A-Phe in them differs from its conformation in CpA-Phe and from conformation of the free A-Phe. In dinucleoside phosphates, conformation of the molecule as a whole is determined more by the structure of the 5'-terminal nucleoside than by that of the 3'-terminal nucleoside³⁸. Therefore C^pA-Phe is not active while CpA^{-H}-Phe is active. The conformation of the nucleoside and its derivatives depends very strongly on the sugar conformation. Small distortions of the non-planar sugar structure change abruptly the orientation of its substituents. These can be seen as the cause of the difference in nucleoside packing in DNA and RNA chains³⁸ determined by the difference in ribose and 2'-deoxyribose conformations. Hence the presence of peptide acceptor activity in A-Phe and A^{-OMe}-Phe and its absence in A^{-H}-Phe becomes clear.

Proceeding from these considerations it is easy to explain the PAA absence in C^pA-Phe whose conformation sharply differs from that of CpA-Phe as well as the PAA absence in U-Phe, G-Phe and a smaller PAA in C-Phe; in the latter case the molecular conformation is determined by the rotation of the base relative to the bond C₁-N³⁹.

As to the reasons for the influence of amino acid residues on the activity of the peptide acceptors, it is possible to presume that the explanation lies in the difference of the molecular conformation depending on the nature of the amino acid residue. Such examples are known. Thus puromycin, as has already been mentioned, has the anti-conformation of the dimethyladenine ring relative to ribose⁴⁰⁻⁴¹ while the 3'-acetyladenosine is in a syn-conformation⁴². We have obtained further confirmation of the importance of the adenine ring position relative to ribose. Phenylalanine esters of 3,5'-anhydroadenosine (aA-Phe) and 8'-bromoadenosine (Br-A-Phe), for which the anti-conformation of the adenine ring is sterically impeded, do not possess PAA at all.

PTC DONOR SITE

The D-site of the PTC is considerably less studied than the A-site since a good model system for its studying was only found in 1973¹⁰. Earlier, only the following was known: the minimum fragment still possessing a high affinity for the D-site is CCA-acylaminoacyl^{43, 44}. The structure of the amino acid residue is also very important. N-formylmethionine esters were more active, N-acetylleucine and N-acetylphenylalanine esters were less active, N-acetylglycine and N-acetylaspartic acid esters were not active⁴³. The amino group of the amino acid residue must be protected⁴³. It was noticed for the peptidyl-tRNA with a different structure of the peptide part that the D-site is not so sensitive to the structure of the remote part of the peptide chain. (Gly)_n-Phe-tRNA with n = 2-9 served as peptide donors, the PDA optimum was achieved at n = 3⁴⁵; there were many other variants of peptidyl-tRNA with natural amino acids^{45, 46}, ureido-derivatives⁴⁷ and compounds containing three-dimensional fluorescent groups⁴⁸. This gives grounds to presume that the peptide is located on the ribosome surface or near the surface.

However a systematic study of the D-site of the PTC began when it was shown that acylaminoacyladenylic acid can be a peptide donor¹⁰. Now it has been shown that the structure of the base and the amino acid residue, the nature of shielding group at the N-terminus of the amino acid and the presence of a free hydroxyl in ribose are very important for the presence of PDA¹¹. For example for pN-(fMet) where N = A, U, G, C and I, it has been determined that pA-(fMet) and pI-(fMet) possess a high PDA, whereas a small activity is registered for pG-(fMet); at the same time pU-(fMet) and pC-(fMet) were not peptide donors. The given data show the difference in the D- and A- sites specificity which is illustrated in Table 2.

Table 2 Comparison of Activity of N-Phe Model Substrates (for the A-site) and pA-(fMet) (for the D-site) Depending on the Nature of the Base in N

Activities	B a s e					
	Ade	Hyp	Cyt	Gua	Ura	
PAA	++	++	+	-	-	
PDA	++	++	-	+	-	

Comparison of the PDA of different N-formylaminoacid esters of the adenylic acid has shown that pA-(fMet) possesses the highest PDA among the preparations studied. In addition the PDA decreases in the series: pA-(fMet), pA-(fLeu), pA-(fPhe). The PDA of pA-(fGly) was not observed. At the same time the adenylic acid esters containing formyl-dipeptide residues have a higher PDA and are comparable by this test with pA-(fMet). The presence of a CH_3 -group in the 2'-hydroxyl in pA^{-OMe}-(fMet) makes this compound as well as its isomer pA^{-OMe}-(fMet) inactive as a donor.

HYPOTHETICAL MODEL OF THE PTC

As mentioned above, an attempt was made to detail the binding sites of the PTC of E. coli ribosomes¹⁴. The main assumption in this hypothesis is that there are two regions of binding for the A-site - the region for the nucleotide component (the binding site of the 3'-terminal adenosine and of the two subsequent cytidines) and the other region which is the "pocket" for the side chains of the amino acid residue. For the D-sites the hypothesis also postulated the binding regions of the whole terminal trinucleotide section of the CCA and a hydrophobic "pocket" of the side chain of hydrophobic peptide amino acids and a hydrophilic cavity for hydrophilic amino acid side chains of the peptide.

Defining more exactly and partly changing this model we formulate here the following considerations.

1. Interactions of both acceptor and donor ends of the aminoacyl-tRNA and peptidyl-tRNA of the PTC are apparently of the protein-nucleic interaction type and not based on nucleic acid-nucleic acid interactions. This is substantiated by a high PAA of A-Phe and a

high PDA of pA-(fMet). If the terminal fragments of both the donor and acceptor bound because of complementary interactions of terminal CCA with the sites of rRNA structures (GGU) as it was supposed in the paper ⁴⁹, the mononucleotide derivatives would not be bound by the corresponding sites since the binding constant one nucleotide-one nucleotide is low for a stable complex. Besides this, in the case of nucleotide-nucleotide interaction, the PAA of C-Phe for the A-Site and the activity of pG-(fMet) for the D-site could not be explained.

2. The A-site of the ribosomal PTC has regions of binding of the constant elements of acceptors - of the heterocyclic base (binding adenine) and the amino group of the amino acid residue. The model substrate possesses the PAA if its molecule in solution has a conformation corresponding to the structure of the PTC A-site by these two regions. The available data lead us to believe that these two sites are sufficient for binding. The role of the two subsequent nucleotides (cytidylic acids) consists only in fixing the terminal adenosine conformation advantageous for the A-site. This can be confirmed by the data on the absence of stimulation by the cytidylic acid or dinucleoside phosphate CpC of the reaction of AcPhe-tRNA with puromycin, catalyzed by ribosomes. If the second or the third nucleotide link from the 3'-end was bound, the reaction would be stimulated due to conformational organization of the PTC A-site.

Several facts speak in favour of binding of the NH_2 -group of the amino acid residue. Aminoacyl-tRNA and CACCA-aminoacyl have a greater affinity for the A-site than free tRNA and CACCA, respectively, or peptidyl-tRNA and CACCA-(acylaminoacyl)⁴. On the other hand, pA-(fMet) only slightly inhibits the acceptor binding¹¹ but effectively inhibits the donor binding. However, even irrespective of these facts, it is difficult to assume free rotation of the NH_2 -group of the substrate after its binding with the A-site of the PTC. This is namely the NH_2 -group which is a direct participant in the reaction and therefore it must be precisely fixed in space. Proceeding from the chemical point of view of the process according to which the NH_2 -group is the nucleophilic component of the reaction of the peptide bond formation, it can be presumed that the functional group of the protein of the PTC A-site must increase the nucleophilic character of the NH_2 -group or at least must not decrease it. Therefore a bond of the hydrogen type must exist in which the protein group would be the proton acceptor and the NH_2 -group of the amino acid residue would be the donor.

Consequently, the NH_2 -group should be bound not by the carboxyl group but more likely by an amino or hydroxy-group, imidazole residue or the CO-group of the peptide bond of the protein forming the PTC. The pH optimum of the transferase reaction near 8.5⁴ corresponds to this prediction. Besides, the high PAA of hydroxypuromycin and hydroxyacyl-tRNA⁵⁰ also agrees with this type of H-bonding with a proton accepted from the hydroxy-group of the peptide acceptor and not the other way round.

3. Two regions of binding of nucleotide components of peptidyl-tRNA are clearly traced for the D-site. First, this is the 3'-terminal adenylic acid and second, the penultimate cytidylic acid residue^{51, 52}. However, it is possible that the peptide residue also binds with the D-site of the PTC and thus contributes to the donor fixation in the D-site. This is confirmed by a considerably higher binding of peptidyl-tRNA containing several peptide residues in the chain in comparison with acylaminoacyl-tRNA⁴⁵ which has been already discussed above. We think that peptide groups take part in such a binding on the part of peptidyl-tRNA.

Thus the general view of the PTC of the bacterial ribosome according to our model is represented in Fig. 3

Fig. 3. Scheme of the PTC of *E. coli* ribosomes
 1. Binding site of the terminal adenine of the acceptor. 2. Binding of the NH_2 -group of the amino acid residue by the protein group X. 3. Binding site of the donor cytosine. 4. Binding site of the donor adenine. 5. Peptide region of binding.

The idea of the importance of the conformation of the model substrate of the ribosomal PTC discussed in this paper outlines the possible approaches to the conformational analysis of the PTC. The puromycin conformation as determined for a crystal agrees with the NMR data showing these general features in solution, this can be taken as a starting point.

ABBREVIATIONS USED.

PTC - Peptidyl Transferase Centre of ribosomes. A-site - the acceptor site and D-site - the donor site. PAA - Peptidyl-acceptor activity. PDA - Peptidyl-donor activity. All the abbreviations of amino acids, peptides, nucleosides, nucleotides and oligonucleotides are in agreement with the recommendations of the IUPAC-IUB. The amino acid residue written after the nucleotide index without additional indications corresponds to the mixture of isomers of 2' and 3' esters of the corresponding amino acid and nucleoside. For example, A-Phe is for 2'(3')-O-phenylalanyladenosine. Substitution in the 3'-position is designated by the amino acid residue written lower and in the 2'-position - written upper. For example, A-^{OME}_{Phe} is for 3'-O-phenylalanyl-2'-O-methyladenosine; A-^{Phe}_H is for 2'-O-phenylalanyl-3'-deoxyadenosine. The NH index between the nucleoside and the amino acid points to the amide type of binding. For example, A-^{OH}_{NHTrp} is for 3'-N-tryptophanylamino-3'-deoxyadenosine. Ade-Gluc-Phe is for 9-(3'-O-phenylalanyl-β-D-glucopyranosyl)-adenine. Structures Ade-HACP-Phe, Ade-Ara-NHPhe, A-^{OH}_{Phe} (or), U*PA-Phe, C*PA-Phe and A*PA-Phe are given in the text. C^{PA}-Phe is the 3'(2')-O-phenylalanine ester of cytidyl-(2'→5') adenosine, f- formyl, N-nucleoside without indication of its actual structure.

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