# THE STIMULATION OF RIBOSOMAL PEPTIDYL TRANSFERASE BY CYTOSINE AND ITS DERIVATIVES

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The ribosomal peptidyl transferase (*E. coli*) catalysed transfer of the fMet residue from pA-fMet to the CpApCpCpA-Phe fragment is stimulated by derivatives of cytosine, 6-azacytosine, and 4-thiouracil. The necessary factor for the stimulatory effect of the particular compound is the ability of the base to form a G : C pair. The substitution at position 1 by a β-anomeric sugar residue bearing at positions 2′,3′ a *cis*-diol grouping in the *ribo* configuration markedly increases the stimulation. The stimulatory effect is strongly enhanced by phosphorylation of the sugar residue at position 5′. All three moieties (the base, the sugar residue, and the phosphate group) must be in a precise mutual orientation.

Peptidyl transferase which catalyses peptide bond formation in protein biosynthesis is an integral part of the 50 S ribosomal subunit. The enzyme contains two substrate binding sites, namely, a donor site ("D-site") for holding the peptidyl-tRNA and an acceptor site ("A-site") for attachment of the aminoacyl-tRNA (ref.[1]). The 3′-terminal sequence of peptidyl-tRNA, CpCpA-acylaminoacyl, is necessary for an effective interaction of the donor substrate with the donor site; CpCpA-fMet is active as a donor substrate, whereas CpA-fMet and A-fMet were reported inactive under the same conditions[2]. However, at a much higher concentration[3], pA-fMet-(2′(3′)-O-(N-formyl-L-methionyl)adenosine 5′-phosphate) exhibits the donor activity. pA-fMet obviously fulfils the principal requirements for a donor substrate and the residues of cytidylic acid in the -CpCpA-fMet appear to take part in the interaction with the binding site of peptidyl transferase.

The transfer reaction with the pA-fMet donor has been recently reported to be strongly stimulated by cytidine 5′-phosphate (CMP). The two components probably interact simultaneously with those parts of the binding site which are otherwise occupied by the cytidine and adenosine residues of the tRNA-acylaminoacyl sequence. In the case of donor substrates longer than pA-fMet, a competition occurs between the free CMP and its residue bound in the terminal sequence; this competition results in a decreased activity of peptidyl transferase in the presence of CMP (ref.[4]).

In the present paper, the functional sites in the molecule of cytidine 5′-phosphate are investigated from the standpoint of their indispensability for the stimulation

of peptidyl transferase with pA-fMet as a donor substrate. Furthermore, properties of the ribosomal site, which is involved in this interaction, are examined.

## EXPERIMENTAL

### Materials

Cytidine, 2′-deoxycytidine, uridine, cytosine, and cytidine nucleotides (as sodium salts) were purchased from Calbiochem (Los Angeles, U.S.A.). L-Phe-4′-$^3$H (21 Ci/mmol) was a product of Radiochemical Centre Amersham (England) 4-Thiouridine was kindly donated by Dr K. H. Scheit (Göttingen, F.R.G.). The β-D-ribonucleosides of 2-pyridinone, 3-pyridazinone, and 6-pyrimidinone were prepared by Dr H. Pischel (Leipzig, G.D.R.); 1-(β-D-ribofuranosyl)-4-amino--6-pyrimidinone was prepared by Dr M. Prystaš of this Institute. If not stated otherwise, the compounds were prepared by methods described earlier (*cf.* Tables for references).

Nucleoside 5′-phosphates were prepared by the reaction of free nucleosides with phosphorus oxychloride in triethyl phosphate[5] with the use of a modification reported earlier[6]. The products were purified by paper chromatography in 2-propanol–concentrated aqueous ammonia–water (7 : 1 : 2) and isolated as ammonium salts. The following compounds were obtained (% yield): *IIb* (64), *IIIb* (66), *Xb* (55), *XIIb* (72), and *XXII* (56). The nucleotide methyl esters *XXXVIII* and 3′-*XXXVI* were obtained by the action of ethereal diazomethane on the corresponding nucleotides (ammonium salts) in aqueous solutions; the 2′-isomer of *XXXVI* was prepared by esterification of cytidine 2′(3′)-phosphate with diazomethane followed by pancreatic RNAse digestion of the product at pH 7·5 and paper chromatography. The 2′-isomer was resistant towards repeated action of the enzyme mentioned. 5′-O-Methoxycarbonylmethylcytidine (*XL*) was prepared by treatment of the carboxylic acid *XXXIX* with diazomethane followed by paper-electrophoretical separation of the neutral product *XL* (0·1M triethylammonium hydrogen carbonate, 20 V/cm).

### Methods

Ribosomes were prepared from *Escherichia coli* B by washing with 0·5M ammonium chloride as described elsewhere[1]. The terminal CpApCpCpA-[$^3$H]Phe fragment was prepared according to Pestka[30]. 2′(3′)-O-(N-formylmethionyl)adenosine 5′-phosphate (pA-fMet) was a gift of Dr A. Krayevsky (Moscow. U.S.S.R.); the substance was prepared by the method for N-acylamino-acylation of 5′-ribonucleotides developed by Gottikh and coworkers[31].

### Transfer Assay

The reaction of pA-fMet with CpApCpCpA-[$^3$H]Phe was carried out under the conditions of fragment reaction[2] and assayed as follows. The reaction mixture contained (prior to the addition of methanol) 0·05M Tris-HCl buffer solution (pH 7·4), 0·4M-KCl, 0·02M magnesium acetate, ribosomes (350 μg), CpApCpCpA-[$^3$H)Phe (150—200 pmol), pA-fMet (200 nmol), and cytosine derivatives in the concentration indicated (total volume, 100 μl). The reaction was initiated by the addition of methanol (100 μl) and the mixture incubated for 90 min at 0°C. The reaction was terminated by the addition of 3M sodium hydroxide (50 μl) in order to hydrolyze the ester bond between oligonucleotide and peptide or aminoacyl residue. After standing for 30 min at 36°C, 5M HCl (400 μl) was added and the fMet-[$^3$H]Phe was extracted from the solution with ethyl acetate (3 ml) and counted. The blank value was obtained by omitting pA-fMet from the incubation mixture.

## RESULTS

In all experiments pA-fMet was as the donor substrate and CpApCpCpA-[$^3$H]Phe as the acceptor substrate of peptidyl transferase.

### *Effect of the Pyrimidine Base Modification*

As shown by data in Table I the stimulatory activity is exhibited not only by CMP but also (though to a lesser extent) by cytidine and cytosine. The effect of the modification was therefore examined on all three types of compounds, namely, alkyl derivatives of bases, nucleosides, and nucleotides.

By substitution of the amino group at position 4 of cytosine, the stimulatory activity of the nucleoside *IIa* and of the 5'-nucleotide *IIb* is decreased to the values of 35% and 68%. The activity is also lowered (28% of the original value) by substitution of the amino group by a hydroxyl with the formation of the 4-hydroxylamino derivative *IVa*. A twofold substitution of the amino group by methyl groups in the series of both nucleosides and nucleotides results in a marked decrease of the stimulatory activity (16% with compound *IIIa* and 13% with compound *IIIb*) to the value corresponding to the parent compound of the pyrimidine series, the 2-pyrimidinone derivative *IXb*, the position 4 of which contains a hydrogen atom. The stimulatory activity is almost completely lost in both series when the nitrogen atom at position 3 is absent (compound *XVa*, *XVb*).

Replacement of the CH group at position 6 of the pyrimidine ring by nitrogen atom (the 6-azacytosine derivatives *XIIa* and *XIIb*) does not affect the stimulatory activity. The activity of both the nucleoside and 5'-nucleotide is almost equal to that of cytosine derivatives. Furthermore, the stimulatory activity is not affected by substitution of the cytosine ring at position 5 by a methyl group (compound *XXXIII*).

All uracil derivatives are entirely inactive even when substituted at position 5 by basic substituents such as the amino (compound *VIa*), the diethylamino (compound *VIIa*) and the diethylaminomethyl group (compound *VIIIa*). On the other hand, all 4-thiouracil derivatives are strongly active; their activity is only a little lower than that of the corresponding cytosine derivatives (1·60 with compound *Xa* and 3·95 with compound *Xb*).

The effect of base modification of the $N^1$-alkyl derivatives (Table II) obeys the same rules as in the series of nucleosides and nucleotides (Table I). The activity of 1-methyl-4-thiouracil (*XXIV*) is comparable with that of 1-methylcytosine (*XVIII*). The position $N^1$ can also be substituted by the chiral 2,3-dihydroxypropyl group; the activity of the (*S*)-enantiomer *XIX* and of $N^1$-methylcytosine (*XVIII*) is roughly equal to that of cytosine. Consequently, the activity is not increased by substitution at position $N^1$, regardless the hydrophilic or hydrophobic nature of the substituent. However, the activity is markedly increased even in the series of non-nucleosidic derivatives if the substituent is negatively charged; *cf.* the high activity of the 3'-

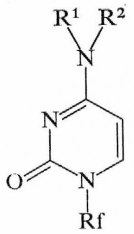
phosphate *XXII* and of $N^1$-carboxymethylcytosine (*XX*). On the contrary, the homologue *XXI* is inactive.

TABLE I

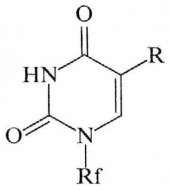
Stimulation Activity of Ribonucleosides and 5'-Ribonucleotides Modified at the Heterocyclic Base

| No | Compound | 0·5 mM | 1 mM |
|---|---|---|---|
| — | — | 1·00[a] | 1·00[a] |
| *Ia* | Cytidine | 1·74 | 2·64 |
| *Ib* | CMP | 2·83 | 4·58 |
| *XVII* | Cytosine | 1·30 | 1·80 |
| *IIa* | $N^4$-Methylcytidine[7] | — | 1·57 |
| *IIIa* | $N^4$-Dimethylcytidine[7] | — | 1·27 |
| *IVa* | $N^4$-Hydroxycytidine[8] | — | 1·46 |
| *Va* | Uridine | 0·96 | 1·06 |
| *VIa* | 5-Aminouridine[9] | 1·16 | 1·17 |
| *VIIa* | 5-Dimethylaminouridine[10,11] | 1·02 | 1·08 |
| *VIIIa* | 5-Dimethylaminomethyluridine | 1·05 | 1·12 |
| *Xa* | 4-Thiouridine | 1·30 | 1·60 |
| *XIa* | 2-(β-D-Rf)-3-pyridazinone[12 b] | 1·18 | 1·29 |
| *XIIa* | 6-Azacytidine[13] | 1·60 | 2·14 |
| *XIIIa* | 1-(β-D-Rf)-6-pyrimidinone[14] | 1·13 | 1·01 |
| *XIVa* | 1-(β-D-Rf)-4-amino-6-pyrimidinone[15,16] | 1·13 | 1·33 |
| *XVa* | 1-(β-D-Rf)-2-pyridinone[12] | 0·96 | 1·00 |
| *XVIa* | 3-(β-D-Rf)uracil[17] | 1·07 | 0·96 |
| *IIb* | $N^4$-Methylcytidine 5'-phosphate | 2·04 | 3·43 |
| *IIIb* | $N^4$-Dimethylcytidine 5'-phosphate | 1·41 | 1·46 |
| *Vb* | UMP | 0·91 | 1·03 |
| *IXb* | 1-(β-D-Rf)-2-pyrimidinone 5'-phosphate[15] | 1·61 | 1·58 |
| *Xb* | 4-Thiouridine 5'-phosphate | 3·03 | 3·95 |
| *XIb* | 2-(β-D-Rf)-3-pyridazinone 5'-phosphate[12] | 1·13 | 1·15 |
| *XIIb* | 6-Azacytidine 5'-phosphate | 3·86 | 3·88 |
| *XIIIb* | 1-(β-D-Rf)-6-pyrimidinone 5'-phosphate[15] | 1·08 | 1·18 |
| *XVb* | 1-(β-D-Rf)-2-pyridinone 5'-phosphate[12] | 1·08 | 1·04 |
| *XXVb* | dCMP | 2·65 | 4·18 |

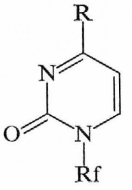
[a] Value of 1·00 corresponds to 882 cpm and refers to the transfer reaction in the absence of an effector; [b] Rf ribofuranosyl residue.



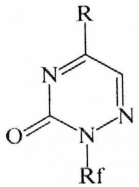
*I*; $R^1 = R^2 = H$
*II*; $R^1 = CH_3$, $R^2 = H$
*III*; $R^1 = R^2 = CH_3$
*IV*; $R^1 = OH$, $R^2 = H$



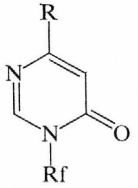
*V*; R = H
*VI*; R = $NH_2$
*VII*; R = $N(CH_3)_2$
*VIII*; R = $CH_2N(C_2H_5)_2$



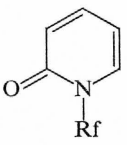
*IX*; R = H
*X*; R = SH



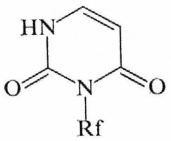
*XI*; R = H
*XII*; R = $NH_2$



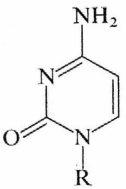
*XIII*; R = H
*XIV*; R = $NH_2$



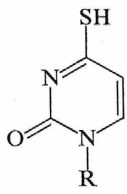
*XV*



*XVI*



*XVII*; R = H
*XVIII*; R = $CH_3$
*XIX*; R = (*S*)—$CH_2CHCH_2OH$ (OH)
*XX*; R = $CH_2COOH$
*XXI*; R = $CH_2CH_2COOH$
*XXII*; R = (*S*)—$CH_2CHCH_2OP(O)(OH)_2$ (OH)



*XXIII*; R = H
*XXIV*; R = $CH_3$

In series *a*: Rf = β-D-ribofuranoside residue,
*b*: Rf = β-D-ribofuranoside 5-phosphate residue.

*Effect of Sugar Modification in Cytidine and Its 5'-Phosphate*

The essential influence of the sugar moiety upon the stimulation activity of the particular compound may be unequivocally inferred from comparison of the corresponding data of cytosine (*XVII*), its $N^1$-methyl derivative *XVIII* and 1-(*S*)-2,3-dihydroxypropyl derivative *XIX* with those of cytidine (*Ia*) and 2'-deoxycytidine (*XXVa*). As indicated in Table II and III, peptidyl transferase is stimulated only by derivatives with a β-glycosidic bond: α-dCMP (*XXXb*) and cytosine (*XVII*) are equally active while dCMP is much more active than cytosine. Replacement of the ribose residue by 2-deoxyribose results in a dramatic decrease of the stimulation activity of the corresponding nucleosides; thus, the activity of 2'-deoxycytidine (*XXVa*) and its 5-methyl derivative *XXXIII* is similar to that of cytosine. On the other hand, the stimulation activity of dCMP (*XXVb*) is comparable with that of CMP. 3'-Deoxycytidine (*XXVII*) and the β-D-xylofuranoside *XXVIII* exhibit the activity similar to that of cytosine alone. The stimulation effect of cytosine arabinofuranosyl derivatives is lower than that of ribofuranosyl derivatives. Thus, araCMP (*XXIXb*) is about half as active as CMP and the effect of araC (*XXIXa*) is about one tenth of cytidine (*i.e.*, the effect is lower than that of cytosine alone). Methylation of the 2'-hydroxylic function of cytidine is accompanied by a similarly decreased activity: the activity

TABLE II

Stimulation Activity of Cytosine and 4-Thiouracil Derivatives Substituted at the Position 1

| No | Compound | 0·5 mM | 1 mM |
|---|---|---|---|
| — | — | 1·00[a] | 1·00A |
| *XVII* | Cytosine | 1·30 | 1·80 |
| *XVIII* | 1-Methylcytosine[18] | | 1·80 |
| *XIX* | 1-(*S*)-(2,3-Dihydroxypropyl)cytosine[19] | | 1·73 |
| *XX* | 1-Carboxymethylcytosine[20] | 1·63 | 2·34 |
| *XXI* | 1-Carboxyethylcytosine[21] | 1·08 | 1·16 |
| *Ia* | Cytidine | 1·74 | 2·64 |
| *XXVa* | 2'-Deoxycytidine | 1·35 | 1·53 |
| *Ib* | CMP | 2·82 | 4·58 |
| *XXII* | 1-(*S*)-(2,3-Dihydroxypropyl)cytosine 3'-phosphate[19] | 1·60 | 2·43 |
| *XXIII* | 4-Thiouracil | 1·02 | 0·97 |
| *XXIV* | 1-Methyl-4-thiouracil[22] | 1·18 | 1·64 |
| *Xa* | 4-Thiouridine | 1·30 | 1·60 |
| *Xb* | 4-Thiouridine 5'-phosphate | 3·04 | 3·95 |

[a] *Cf.* Table I.

of the nucleoside *XXVIa* amounts to about 25% of cytidine activity while 2'-O-methylcytidine 5'-phosphate (*XXVIb*) acts as inhibitor of the reaction. Methylation of the 5'-hydroxylic function of cytidine (compound *XXXI*) displays a considerably lower effect on the stimulation activity.

*Effect of Phosphorylation of Nucleosides*

The stimulation activity of nucleosides (Table IV) is markedly enhanced by phosphorylation of their 5'-hydroxylic function: by 118% with cytidine. 600% with cytosine-arabinoside, 150% with 6-azacytidine, 400% with 4-thiouridine, and 500% with 2'-deoxycytidine. However, the effect arises with those nucleosides only, which are active *per se* (both uridine and uridine 5'-phosphate are inactive and 2'-O-methylcytidine 5'-phosphate as well as L-CMP are inhibitors of peptidyl transferase). The isomeric cytidine 2'(3)'-phosphate (*XXXV*) is virtually inactive.

Introduction of a di- or triphosphate residue into the position 5' of cytidine does not increase the stimulation activity of CDP and CTP when compared with that of CMP. Cytidine 5'-phosphate methyl ester (*XXXVIII*) and cytidine 5'-phosphite (*XXXVII*)

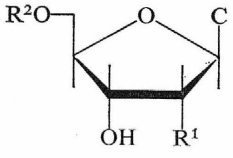
TABLE III

Stimulation Activity of Cytidine and CMP Derivatives Modified at the Sugar Moiety

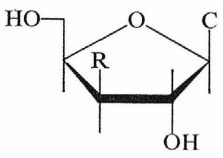
| No | Compound | 0·5 mM | 1·0 mM |
|---|---|---|---|
| — | — | 1·00[a] | 1·00[a] |
| *Ia* | Cytidine | 1·74 | 2·64 |
| *XXVa* | 2'-Deoxycytidine | 1·35 | 1·53 |
| *XXVIa* | 2'-O-Methylcytidine[24] | 1·17 | 1·42 |
| *XXVII* | 3'-Deoxycytidine[25] | 1·33 | 1·47 |
| *XXXVIII* | 1-(β-D-Xylofuranosyl)cytosine | 1·28 | 1·69 |
| *XXIXa* | 1-(β-D-Arabinofuranosyl)cytosine[23] | | 1·18 |
| *XXXa* | 2'-Deoxy-α-cytidine[26] | 1·38 | 1·52 |
| *XXXI* | 5'-O-Methylcytidine[24] | 1·78 | 2·49 |
| *XXXII* | 2',3',5'-Tri-O-methylcytidine[24] | 1·13 | 1·15 |
| *XXXIII* | 5-Methyl-2'-deoxycytidine | 1·00 | 1·36 |
| *Ib* | Cytidine 5'-phosphate | 2·83 | 4·48 |
| *XXVb* | 2'-Deoxycytidine 5'-phosphate | 2·65 | 4·18 |
| *XXVIb* | 2'-O-Methylcytidine 5'-phosphate[6] | 0·36 | 0·28 |
| *XXIXb* | 1-(β-D-Arabinofuranosyl)cytosine 5'-phosphate[6] | 2·01 | 2·40 |
| *XXXb* | 2'-Deoxy-α-cytidine 5'-phosphate[26] | 1·23 | 1·65 |
| *XXXIVb* | L-Cytidine 5'-phosphate[27] | 0·79 | 0·51 |

[a] *Cf.* Table I.

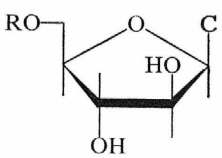
are less active than cytidine. However, the activity is substantially increased by substitution of cytidine 5'-hydroxylic function by a carboxymethyl group: 5'-O-carboxymethylcytidine (*XXXIX*) is more active than CMP while the activities of the araC 5'-O-carboxymethyl derivative *XLI* and of araCMP (*XXIXb*) are almost identical. Esterification of the above highly active compound *XXXIX* with the formation of 5'-O-methoxycarbonylmethylcytidine (*XL*) results in a loss of both the negative charge and the stimulation.



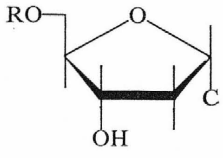
*XXV*; $R^1 = H$
*XXVI*; $R^1 = OCH_3$
*a*: $R^2 = H$
*b*: $R^2 = P(O)(OH)_2$



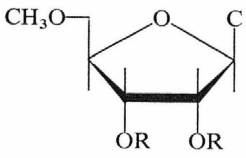
*XXVII*; R = H
*XXVIII*; R = OH



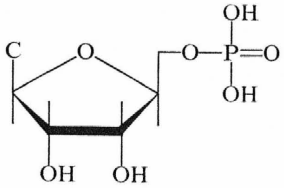
*XXIXa*: R = H
*XXIXb*; $R = P(O)(OH)_2$



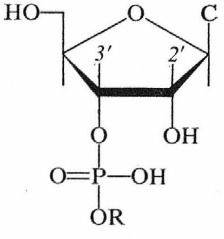
*XXXa*: R = H
*XXXb*: $R = P(O)(OH)_2$



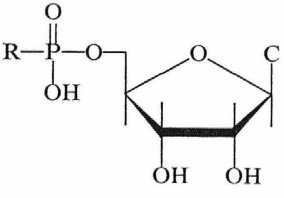
*XXXI*; R = H
*XXXII*; $R = CH_3$



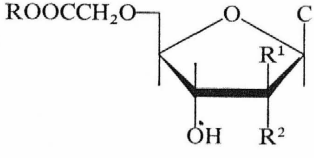
*XXXIV*



*XXXV*; R = H
*XXXVI*; $R = CH_3$



*XXXVII*; R = H
*XXXVIII*; $R = OCH_3$



*XXXIX*; $R^1 = R = H$, $R^2 = OH$
*XL*; $R^1 = H$, $R^2 = OH$, $R = CH_3$
*XLI*; $R = R^2 = H$, $R^1 = OH$

In formulae *XXV*–*XLI* C = cytosine residue.

## DISCUSSION

The interaction of the cytosine base with the ribosome may be regarded as the essential step governing the stimulation of peptidyl transferase by monomeric derivatives. As demonstrated by various chemical modifications of the base, its pseudo-aromatic character plays only a marginal role in the stimulation effect; it is also supported by a low, but reproducible stimulation activity of the 2-pyrimidinone derivative *IXb*, bearing a hydrogen atom instead of the amino group at the position 4. The effect is obviously induced by hydrogen bonding between the base of the effector and some ribosomal component. Therefore, the necessary requirement is the simultaneous presence of a proton-donor group at position 4, a proton-acceptor group at position 2, and a nitrogen atom at position 3 (*cf.* the activity of the cytidine derivatives *I*, 2-pyrimidinone derivatives *IX*, and 2-pyridinone derivatives *XV*). These requirements correspond to the Watson–Crick base-pairing rules for cytosine–guanine (C : G) pair[32]; CMP and other stimulating derivatives can be thus assumed to interact specifically with the guanylic acid residue in that part of rRNA which belongs to the donor site of peptidyl transferase[33–36].

TABLE IV
Stimulation Activity of Cytosine Nucleosides Bearing an Electronegatively Charged Group

| No | Compound | 0·5 mM | 1 mM |
|---|---|---|---|
| — | — | 1·00[a] | 1·00[a] |
| *Ia* | Cytidine | 1·74 | 2·64 |
| *Ib* | CMP | 2·83 | 4·58 |
| | CDP | 3·14 | 4·87 |
| | CTP | 3·56 | 3·76 |
| *XXXV* | Cytidine 2′(3′)-phosphate | 1·00 | 1·00 |
| 2′-*XXXVI* | Cytidine 2′-phosphate methyl ester | 1·06 | 0·97 |
| 3′-*XXXVI* | Cytidine 3′-phosphate methyl ester | 1·06 | 1·00 |
| *XXXVII* | Cytidine 5′-phosphite[28] | 1·36 | 1·79 |
| *XXXVIII* | Cytidine 5′-phosphate methyl ester | 1·42 | 2·28 |
| *XXXIX* | 5′-O-Carboxymethylcytidine[29] | 3·65 | 5·05 |
| *XL* | 5′-O-Methoxycarbonylmethylcytidine | 1·12 | 1·28 |
| *XXIXa* | 1-(β-D-Arabinofuranosyl)cytosine | | 1·18 |
| *XLI* | 5′-O-Carboxymethyl-1-(β-D-arabinofuranosyl)-cytosine[29] | 2·02 | 2·32 |

[a] *Cf.* Table I.

This type of interaction is also supported by the high activity of 6-azacytosine derivatives *XIIa,b* which are almost equivalent to cytosine derivatives with regard to the steric arrangement of the molecule and the ability of forming the G : $n^6$C pair[37]. Similarly, the 4-thiouracil derivatives, bearing a proton-donor -SH group at position 4 and a keto group at position 2, can form a non-classical Watson–Crick pair with guanine; such a possibility has been proposed for some interactions of 4-thiouridine in tRNAs (ref.[38]). Consequently, the 4-thiouracil derivatives also stimulate the activity of peptidyl transferase. Substitution of the amino group at position 4 of cytosine by an alkyl or hydroxyl group is accompanied by a decreased ability to form hydrogen bonds whereas its disubstitution entirely excludes the involvement of this group as a proton-donor. Thus, the monosubstitution results in a decrease and the disubstitution in a suppression of the stimulation activity of cytosine derivatives. Uracil derivatives cannot form complexes with guanine and are therefore entirely inactive.

The formation of a C : G pair requires also the simultaneous presence of a proton acceptor function, namely a carbonyl group at position 2. This condition is fulfilled in cytosine and 4-thiouracil derivatives but not in the isomeric 4-amino-6-pyrimidinone derivative *XIV*, the carbonyl group of which is not part of a carbamide grouping. The third interaction occurring in the G : C pair is the hydrogen bond between the $N^1$-H grouping of guanine and the unshared electron pair of the ring nitrogen at position 3. The formation of this bond and the presence of a proton-accepting group at position 2 impart a weak activity to the 2-pyrimidinone derivative *IXb*. On the other hand, no activity is observed with 1-(β-D-ribofuranosyl)-2-pyridinone (*XVa*) and its 5′-phosphate *XVb* (both lacking the $N^3$ nitrogen atom of the pyrimidine ring) and with the aza analogues *XIa,b*. The isomers of compounds *IX*, namely 1-(β-D-ribofuranosyl)-6-pyrimidinone (*XIIIa*) and its 5′-phosphate *XIIIb* carry (similarly to the 4-amino derivative *XIVa*) a carbonyl function at position 6 which is oriented unfavourably with respect to the $N^3$ nitrogen atom; therefore, the contribution of these two functions (observable with compound *IXb*) is lost and the derivatives *XIIIa,b* are entirely inactive. Similarly, the occurrence of the 1-NH group in 3-(β-D-ribofuranosyl)uracil (*XVIa*) excludes the possibility of the base-pair formation with guanine.

In addition to this essential principle of the heterocyclic base interaction with the ribosome by Watson–Crick base pairing, further effects depending on the character of the substituent at position $N^1$ of the cytosine base may affect the stimulation of peptidyl transferase activity. The mere substitution at position 1 of cytosine does not enhance the stimulation activity; it follows from comparison of cytosine (*XVII*), 1-methylcytosine (*XVIII*), and (*S*)-1-(2,3-dihydroxypropyl)cytosine (*XIX*). The stimulation activity increases on substitution of the position 1 by a group bearing an electronegative charge, *i.e.* compounds *XX* and *XXII*. In these simple cytosine derivatives, the amino group at position 4 is obviously influenced by the I-effect of 1-carboxy-

alkyl group which drops with the growing chain (*cf.* compound *XXI*) or by a long-range effect in compound *XXII*.

The sugar moiety at position 1 of cytosine nucleosides involves both a chirality effect (essential for interactions with chiral partners), and stabilisation of some conformation, and, finally, presence of several hydroxylic groups. When the hydroxylic function at position 2′ of the sugar residue in cytidine is absent or methylated, the conformation is hardly changed, only the stereochemistry of the sugar ring (ring-puckering) is somewhat affected[39]. However, in the series of nucleosides and nucleotides, both these modifications result in a loss or decrease of the stimulation activity. A lower activity may also be observed in the case of 3′-deoxycytidine (*XXVII*) and the β-D-xylofuranoside *XXVIII*, both of which bear the 2′-hydroxylic function in the *ribo* configuration. However, 5′-O-methylcytidine (*XXXI*) exhibits approximately the same activity as cytidine. The activity appears to increase in the presence of a *cis*-2′,3′-diol system in the molecule of the effector. The data observed with the araC derivatives *XXIXa,b* are in accordance with this observation; the changed conformation of the nucleoside caused by the configuration of the 2′-hydroxylic group might be also responsible for the decrease of the stimulation activity in compounds *XXIXa,b* (ref.[39]).

The stimulation activity of cytosine nucleosides is strongly affected by phosphorylation. The presence of a 5′-phosphate grouping often results in such an increase of the activity which covers differences due to base or sugar alteration including the replacement of the latter by the 2,3-dihydroxypropyl group. Since CMP, CDP, and CTP exhibit an almost equal activity, the effect appears to be independent upon an additional increase of the virtual charge. The lowering of this charge by esterification of the nucleotide or by introduction of a phosphorous acid residue suppresses the stimulatory activity below the cytidine value (compounds *XXXVI* and *XXXVII*). However, the substitution of the 5′-hydroxylic function of cytidine by a carboxymethyl group (compound *XXXIX*) strongly enhances the stimulation activity. Although the carboxymethyl substituent is capable of a single dissociation only, the gauche-gauche configuration of the 5′-hydroxylic function[39] obviously facilitates a suitable mutual orientation of the substituent group and the heteroaromatic system. Owing to a longer chain in comparison with compounds *XXXVII* and *XXXVIII*, the monoanion of compound *XXXIX* may assert itself to a greater extent.

Cytidine 2′- or 3′-phosphates do not exhibit any effect on the transfer reaction. Consequently, the mutual orientation of the characteristic groups of the base, the *cis*-2′,3′-diol grouping of the sugar residue, and the negatively charged group (the phosphate group) determine the optimal steric arrangement of the effector molecule and its orientation to the binding site. This requirement is not realised in 2′-deoxy-α-cytidine (*XXXa*) and its 5′-phosphate *XXXb*, the stimulation activity of which equals that of cytosine alone. Comparison of the activity data for CMP and L-CMP (*i.e.*, compounds with the same steric arrangement but a reversed chirality) indicates

that the partner of CMP in the complex formation must be a chiral molecule or system (rRNA) which interacts with the whole, *i.e.* the chiral molecule of the nucleoside effector[40].

The stimulation effect on peptidyl transferase might be due to a previous interaction of the effector with the donor substrate pA-fMet, followed by increase of substrate attachment in the binding site. The donor site appears to be occupied by both the partial substrates (pA-fMet and the effector); the increase of activity in comparison with pA-fMet alone might be explained by the induced-fit theory[41]. Finally, it seems quite plausible to assume that the cytidylic acid residues of the terminal tRNA sequences interact with peptidyl transferase in a similar way, *i.e.* by Watson–Crick C : G pair formation with a part of rRNA situated in the donor site.

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