THE EFFECT OF THE AMINOACYL-4-AMINOHEXOSYL-CYTOSINE GROUP OF ANTIBIOTICS ON RIBOSOMAL PEPTIDYL TRANSFERASE

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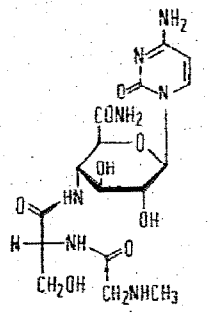
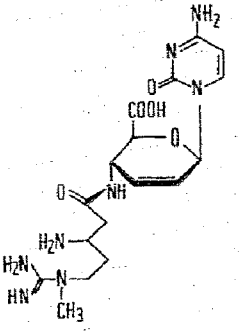
1. Introduction

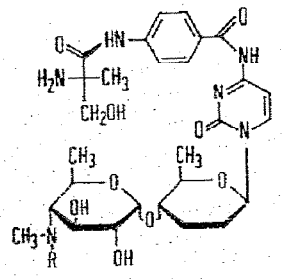
Extensive studies with bacterial and mammalian ribosomal systems have provided a fund of information with respect to the inhibition of protein biosynthesis at the peptide chain elongation stage by gougerotin (1), blasticidin S (2) and amicetin (3) [1]. Within this group of antibiotics, which in fact show very similar inhibitory patterns, and which, on the basis of their gross structural features, may be designated the *aminoacyl-4-amino-hexosyl-cytosine* group of 50 S inhibitors, very little is known about structure–activity relationships, although first correlative indications may be extracted from the biological evaluation of some analogues of blasticidin S [2] and of gougerotin [3]. In our pursuit of further assessing steric and functional group requirements for inhibitory activity within this group of antibiotics, we initiated an evaluation of the effects of bamicetin (4) and plicacetin (5) on ribosomal peptidyl transferase. Though these antibiotics (4 and 5) have been surmised [1, 4] to biologically belong into the same group with 1–3 as of now, only bacterial screening data [5, 6] are available.



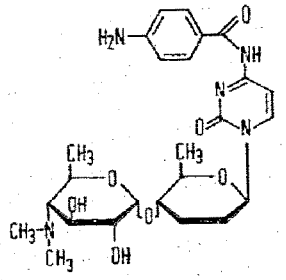
1: GOUGEROTIN



2: BLASTICIDIN S



3 R=CH_3: AMICETIN
4 R=H: BAMICETIN



5: PLICACETIN

2. Materials and methods

Ribosomes were prepared from *Escherichia coli* B as described elsewhere [7]. Gougerotin was purchased from Calbiochem USA. Blasticidin S was a gift from Dr. H. Seto of the Institute of Applied Microbiology, the University of Tokyo; amicetin, bamicetin and plicacetin were gifts from Dr. T.H. Haskell, Parke-Davis, Ann Arbor, Michigan.

2.1. *Transfer assay with $(Lys)_n$-tRNA*

The transfer of lysine peptides from $(Lys)_n$-tRNA to puromycin was measured according to Rychlik et al. [7]. The incubation lasted 40 min at 35°. The

samples were precipitated with 5% trichloracetic acid, filtered and counted [7].

2.2. *Transfer assay with AcPhe-tRNA*

The transfer of the AcPhe-residue from AcPhe-tRNA to puromycin was measured according to [7]. After a 30 min incubation period at 35°, the AcPhe-puromycin formed was extracted into ethylacetate [8] as modified by Monro et al. [9].

2.3. *Transfer assay with the CACCA-(AcLeu) fragment*

The transfer of the AcLeu-residue from the CACCA-(AcLeu) fragment to puromycin was measured according to Monro et al. [9].

2.4. *Assay of the CACCA-(AcLeu) substrate binding to the donor site*

The procedure of Celma et al. [10] was used.

2.5. *Assay of the CACCA-(Phe) substrate binding to the acceptor site*

The procedure of Pestka [11] was used.

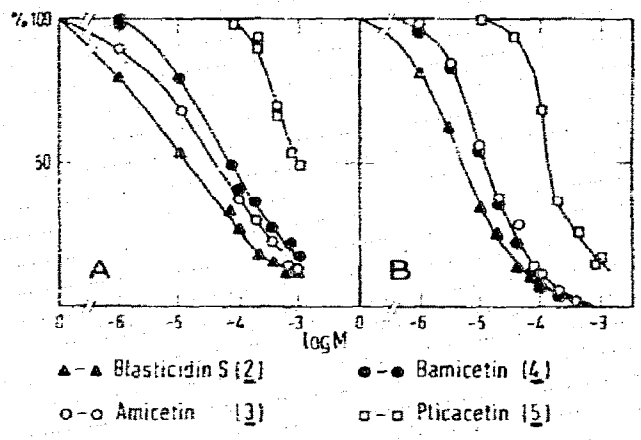


Fig. 1. The effect of blasticidin S, amicetin, bamicetin and plicacetin (2–5) on the transfer of lysine peptides from $(Lys)_n$-tRNA (A) and of the AcPhe-residue from tRNA (B) to puromycin. The reaction mixture contained AcPhe-tRNA (20 μg, 2100 cpm) or $(Lys)_n$-tRNA (10 μg, 2800 cpm). In control experiments 65% of the AcPhe-residue from added AcPhe-tRNA and 48% of lysine peptides from added $(Lys)_n$-tRNA were transferred to puromycin. Log M, concentration of 4-aminohexose pyrimidine nucleosides. %, AcPhe-puromycin or $(Lys)_n$-puromycin formed as percentage of control without inhibitor.

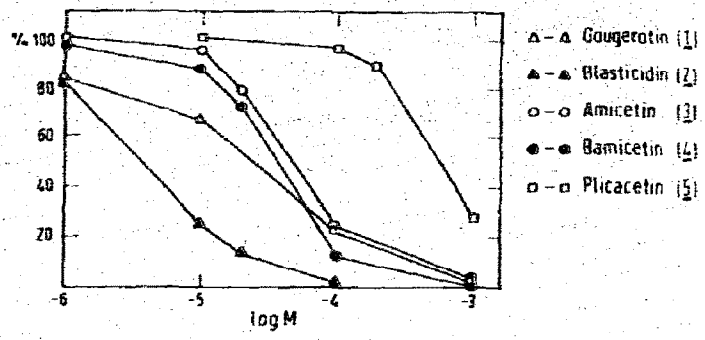


Fig. 2. Effect of the antibiotics 1–5 on the fragment reaction of AcLeu-pentanucleotide with puromycin. Reaction mixtures contained ribosomes (100 μg protein) and Ac[^{14}C]Leu-pentanucleotide (1100 cpm); other components of the reaction mixture, and procedure were described elsewhere [9]. Log M, concentration of antibiotics (calculated on the basis of final volume after addition of methanol). %, AcLeu-puromycin formation as % control without inhibitors (about 900 cpm transferred).

3. Results

3.1. *The effect of nucleoside antibiotics 1–5 on the puromycin reaction with AcPhe-tRNA or $(Lys)_n$-tRNA as donor substrates*

The effect of these antibiotics on peptidyl transfer from intact peptidyl-tRNA was tested with two donor substrates, $(Lys)_n$-tRNA (fig. 1A) and AcPhe-tRNA (fig. 1B). Puromycin reaction with these donors occurs only in the presence of 70 S ribosomes and of the appropriate messenger RNA. All antibiotics tested inhibited the transfer of the acylaminoacyl residue to puromycin. Blasticidin S was the most effective, plicacetin was almost ineffective. The inhibitory effect decreased in the sequence blasticidin S, gougerotin, amicetin = bamicetin (1–5, respectively) and was found to depend on the nature of the donor substrate. With AcPhe-tRNA as the donor, complete inhibition of the AcPhe-transfer to puromycin was observed at 10^{-3} M conc. of amicetin or bamicetin, plicacetin showing only about 1% of their activity (fig. 1B). With $(Lys)_n$-tRNA as donor, the antibiotics were less active (fig. 1A), such that complete inhibition of the puromycin reaction was not reached at 10^{-3} M concentrations. Plicacetin again showed only about 1% of the activity of amicetin or bamicetin.

Table 1
The effect of antibiotics on the CACCA-[^{3}H]Phe and CACCA-(Ac[^{14}C]Leu) binding to ribosomes.

	Conc. (M)	CACCA-[^{3}H]Phe		CACCA-(Ac[^{14}C]-Leu)	
		(cpm)	(%)	(cpm)	(%)
Control	–	702	100	530	100
Amicetin	10^{-4}	405	58	578	109
	10^{-3}	321	46	620	117
Bamicetin	10^{-4}	492	70	680	128
	10^{-3}	303	43	690	130
Plicacetin	10^{-4}	747	106	584	110
	10^{-3}	609	87	604	114
Blasticidin S	10^{-4}	588	84	1216	229
	10^{-3}	486	69	1300	245
Gougerotin	10^{-4}	611	87	753	142
	10^{-3}	386	55	870	164

Assay of CACCA-Phe binding was determined according to Pestka [5]. Reaction mixtures containing CACCA-[^{3}H]Phe (3520 cpm), 70 S ribosomes (6 A_{260} units) and 20% (v/v) ethanol were incubated at 24° for 20 min. Assay of CACCA-(Ac[^{14}C]Leu) binding was determined according to Celma et al. [4]. The incubation mixtures containing CACCA-Ac[^{14}C]-Leu (3380 cpm) and 70 S ribosomes (9 A_{260} units) were incubated at 0° for 40 min.

3.2. *The effect of nucleoside antibiotics 1–5 on the fragment reaction with CACCA-(AcLeu) as donor substrate*

The nucleoside antibiotics inhibited the formation of a new peptide bond also in the simplest system tested, in the fragment reaction (fig. 2). The inhibition decreased in the same sequence as in the case of the puromycin reaction. Blasticidin S was much more effective than the other antibiotics, whereas in the preceding systems containing 70 S ribosomes, messenger RNA and intact acylaminoacyl-tRNA, this difference was less pronounced.

3.3. *The effect of 4-aminohexose pyrimidine nucleosides on the binding of substrates to the donor and acceptor site of peptidyl transferase*

All nucleoside antibiotics (1–5) that inhibited peptidyl transferase activity are capable of intervening with the binding of substrates to peptidyl transferase (table 1). Blasticidin S and gougerotin increased markedly the binding of the donor substrate to the donor site and decreased the binding of acceptor substrate to the acceptor site. Amicetin and bamicetin, at comparable concentrations, however, exhibited only a very poor increase in the substrate binding to the donor site, but markedly decreased the binding of substrate to the acceptor site. Plicacetin had only a slight effect on either site. Yet despite of their interference the binding of the acceptor substrate to the acceptor site of peptidyl transferase, none of these antibiotics (1–5) acted as an acceptor substrate or had any puromycin-like action.

4. Discussion

As might be expected from the close resemblance of their structures which differ only in an N-methyl group in the disaccharide portion (cf. formulae 3 and 4), amicetin and bamicetin show practically identical inhibitory patterns as to their interference with transpeptidation. They inhibit strongly substrate binding to the acceptor site and cause only a slight increase of donor substrate binding, thus it seems not inappropriate to bestow the wealth of information available for amicetin [1] to bamicetin as well. Plicacetin (5), however, lacking the α-methylseryl portion of the amicetin molecule, is nearly inactive as an inhibitor of peptidyl transferase, despite its reported [5] antibacterial activity. This strongly indicates that an aminoacyl moiety with its steric and/or functional group factors is one of the structural features essential for interference with the transpeptidation irrespective of the inability of these antibiotics to accept peptidyl residues onto their aminoacyl part.

As compared with amicetin and bamicetin, the effect of gougerotin (1) and blasticidin S (2) on peptidyl transferase is very similar. Both (1 and 2) strongly inhibit the binding of the acceptor substrate to peptidyl transferase, conceivable by competing with the acceptor substrate for the corresponding binding site. When rationalizing this behaviour in terms of structure–activity relationships, particularly since the uracil analogue of blasticidin S is practically inactive [2], it lies at hand to ascribe an important role in inhibition to the cytosine moiety of 1 and 2 (and probably as well of 3 and 4, although they are differently substituted). This suggestion is consistent with recent

findings on the effect of 2′(3′)-*O*-aminoacyl cytidines and certain aminoacyl-CpA-derivatives on peptidyl transferase, that strongly point towards a specific binding region not only for the last nucleobase (adenine), but also for the penultimate one (cytosine). It is possible, therefore, that the antibiotics 1—4 can participate with their cytosine nucleobase in interactions which usually involve the binding of the penultimate cytosine residue of the 3′-oligonucleotidylic end (CpCpA-terminus) of an aminoacyl-tRNA. Consequently, if the aminoacyl portion of these antibiotics is one of the essential structural features for inhibition of transpeptidation, cf. above, the presence of a cytosine nucleobase appears to be another.

In addition to their action on the acceptor site, blasticidin S and gougerotin also interfere with the donor site by markedly increasing the binding of the donor substrate to ribosomes, whilst amicetin and bamicetin are mostly devoid of this property. At present we are unable to explain these observations. Yet, it is tempting to speculate that the stimulation of donor substrate binding by 1 and 2 could be caused by formation of a stable inactive complex ribosome—antibiotic—donor substrate — similar to the complexes formed with sparsomycin [13] — whereas the amicetin type antibiotics are incapable of doing so. Another possibility is that the antibiotics with a "free" cytosine nucleobase (1 and 2, respectively, as compared to 3 and 4, where the cytosine amino group is acylated) could be capable of opening binding sites that are inaccessible to the approach of the donor substrate under natural conditions.

Acknowledgements

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