THE EFFECT OF GOUGEROTIN ANALOGUES ON RIBOSOMAL PEPTIDYL TRANSFERASE

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1. Introduction

Gougerotin (I), a dipeptidyl pyrimidine nucleoside antibiotic, has been found to specifically inhibit the step of protein biosynthesis which is catalyzed by ribosomal peptidyl transferase [1–5]. Since three gougerotin analogues have been synthesized recently [6], 1-[3-(sarcosyl-D-seryl-)amino-3-deoxy-β-D-glucopyranosyl-)uracil (II, cf. fig. 1), 1-[3-(sarcosyl-D-seryl-)amino-3-deoxy-β-D-glucopyranosyl-)cytosine (III) and 1-[4-(sarcosyl-D-seryl-)amino-4-deoxy-β-D-glucopyranosyl-)cytosine (IV), we made an attempt to characterize the mechanism of action of gougerotin (I) and its analogues (II–IV) on the activity of ribosomal peptidyl transferase. The results are presented below, together with a discussion of relationships between structures I–IV and their inhibitory activity.

2. Materials and methods

Ribosomes were prepared from *Escherichia coli* B as described elsewhere [7]. Gougerotin was purchased from Calbiochem USA. The gougerotin analogues were prepared according to Lichtenthaler et al. [6].

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

The transfer of lysine peptides from $(Lys)_n$-tRNA to puromycin was measured according to Rychlík et al. [7]. The incubation lasted 40 min at 35°. The samples were precipitated with 2.5% trichloroacetic acid, filtered and counted.

2.2. *Transfer assay with AcPhe-tRNA*

The transfer of the AcPhe-residue from AcPhe-tRNA to puromycin was measured according to [7].

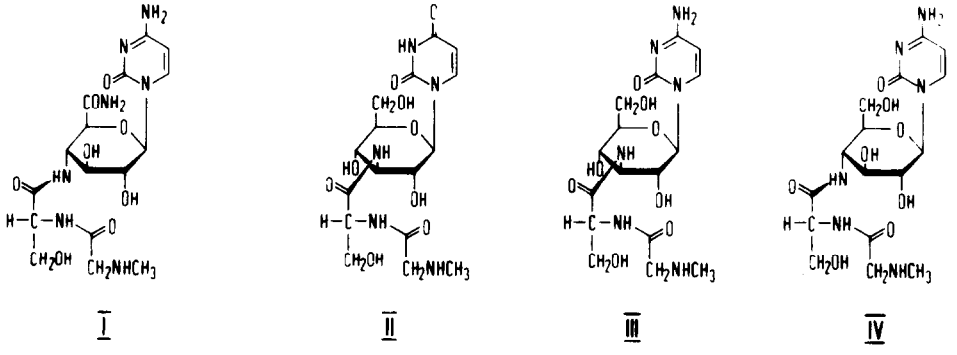


Fig. 1. Formulas of gougerotin (I) and its analogues (II–IV).

After a 30 min incubation period at 35°, the AcPhe–puromycin formed was extracted into ethyl acetate [8] as modified by Monro, Černá and Marcker [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].

For assay of CACCA–(AcLeu) binding to the donor site, the procedure of Celma, Monro and Vazquez [4] was used, whereas CACCA–(Phe) binding to the acceptor site was assayed according to Pestka [5].

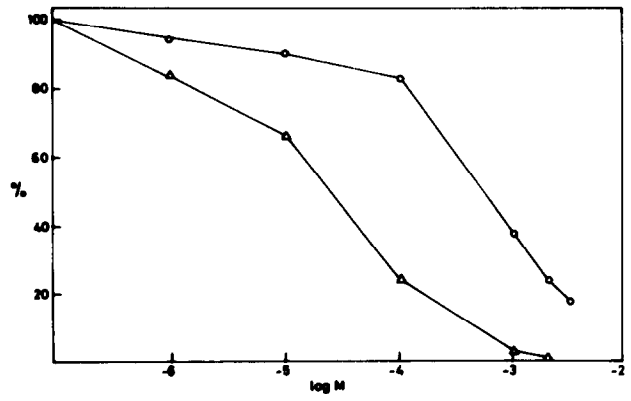
3. Results

3.1. *The effect of gougerotin on the transfer reaction*

Gougerotin inhibits the activity of ribosomal peptidyl transferase. The most pronounced effect of gougerotin was observed in the case of the fragment reaction, i.e. the transfer of the AcLeu residue from the CACCA–(AcLeu) fragment to puromycin (fig. 2). The transfer of the AcPhe residue or of lysine peptides to puromycin which takes place on the 70 S ribosomes with intact molecules of either AcPhe-tRNA or $(Lys)_n$-tRNA and with appropriate messenger RNA, was less sensitive towards gougerotin action (fig. 3). In this case gougerotin caused a 50% inhibition of transferase activity at a concentration higher by one order of magnitude than in the case of inhibition of the fragment reaction. The time course of the effect of gougerotin on the transfer of lysine peptides to puromycin is shown in fig. 4. The time course of its effect on the transfer of the AcLeu-residue from the CACCA–(AcLeu) fragment is presented in fig. 5.

3.2. *The effect of gougerotin analogues*

Only one of the gougerotin analogues (IV in fig. 1) inhibited the activity of peptidyl transferase (fig. 2 and 3). It had a weaker effect than gougerotin. Fig. 4 shows the time course of the effect of the gougerotin



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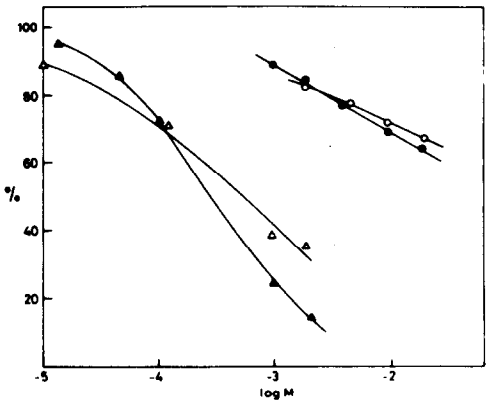

(3)

Fig. 2. Effect of gougerotin analogue (IV) and of gougerotin on the fragment reaction of AcLeu–pentanucleotide with puromycin. Reaction mixtures contained ribosomes (100 μg protein), and about 0.1 μmole AcLeu–pentanucleotide (1200 cpm); other components of the reaction mixture, conditions and procedure were described in [9]. The amount of formed AcLeu–puromycin was determined as the difference between radioactivity extracted into ethyl acetate after incubation with puromycin. Log M, concentration of gougerotin derivative or of gougerotin (concentration calculated on basis of final volume after addition of methanol). %, AcLeu–puromycin formation as % of control without inhibitor (about 1000 cpm transferred); △—△, gougerotin, ○—○, analogue (IV).

Fig. 3. The effect of gougerotin analogue (IV) and of gougerotin on the transfer of lysine peptides from $(Lys)_n$-tRNA and of AcPhe-residue from AcPhe-tRNA to puromycin. The reaction mixture contained $(Lys)_n$-tRNA (10 μg, 1880 cpm) or AcPhe-tRNA (20 μg, 1920 cpm). In control experiments 65% of lysine peptides from added $(Lys)_n$-tRNA and 50% of AcPhe-residue from added AcPhe-tRNA were transferred to puromycin. Log M, concentration of gougerotin analogue or of gougerotin. %, $(Lys)_n$-puromycin or AcPhe–puromycin formed as percentage of control without inhibitor. ▲—▲, AcPhe-tRNA + gougerotin; △—△, $(Lys)_n$-tRNA + gougerotin; ●—●, AcPhe-tRNA + analogue (IV); ○—○, $(Lys)_n$-tRNA + analogue (IV).

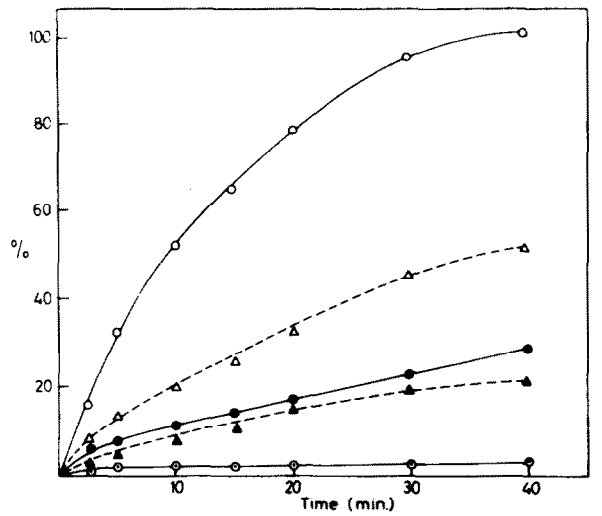

Fig. 4. The time course of the effect of gougerotin analogue (IV) and of gougerotin on the fragment reaction of AcLeu–pentanucleotide with puromycin. Experimental conditions as in fig. 2. %, amount of AcLeu-residue transferred to puromycin as percentage of AcLeu–pentanucleotide added. ○—○, CACCA–(AcLeu) + puromycin; ●—●, the effect of 6.6×10^{-5} M gougerotin; ⊙—⊙, the effect of 10^{-3} M gougerotin; △- - -△, the effect of 6.6×10^{-4} M analogue (IV); ▲- - -▲, the effect of 2×10^{-3} M of derivative (IV).

analogue (IV) on the transfer of lysine peptides to puromycin, fig. 5 its effect on the transfer of AcLeu-residue from the CACCA–(AcLeu) fragment. The gougerotin analogues II and III had no inhibitory effect on peptidyl transferase at any concentration tested.

Neither gougerotin itself, nor its analogues were acceptors of the transferred acylaminoacyl residue, i.e. the inhibition of peptidyl transferase was not caused by a puromycin-like action. This finding is in agreement with the absence of a free α-NH_2 or α-OH group in the compounds studied.

3.3. *The effect of gougerotin and its analogue on the CACCA–(AcLeu) binding to the donor site*

The gougerotin analogue (IV) which inhibited the transfer of the peptide moiety to puromycin, stimulated the binding of the donor substrate CACCA–(AcLeu) to the donor site (table 1). It has a similar, although lower, effect as the parent compound gougerotin.

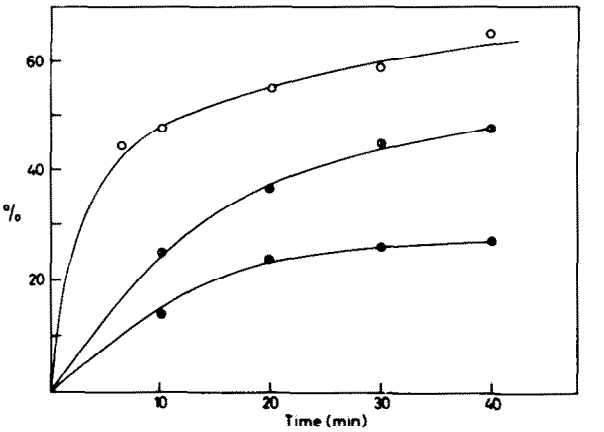

Fig. 5. The time course of the effect of gougerotin analogue (IV) and of gougerotin on the transfer of lysine peptides from $(Lys)_n$-tRNA to puromycin. Experimental conditions as in fig. 3. %, amount of lysine peptides transferred to puromycin as percentage of $(Lys)_n$-tRNA added; time in minutes. ○—○, puromycin 10^{-4} M; ⊙—⊙, puromycin 10^{-4} M + analogue (IV) 10^{-2} M; ●—●, puromycin 10^{-4} M + gougerotin 10^{-3} M.

3.4. *The effect of gougerotin and its analogue on the CACCA–(Phe) binding to the acceptor site*

The gougerotin analogue IV inhibited the binding of the acceptor substrate CACCA–(Phe) to the acceptor site of peptidyl transferase (table 1). In this case the effect of the analogue was also lower than the effect of gougerotin.

4. Discussion

The gougerotin analogues, which were tested as to their effect on peptidyl transferase, differ from the parent compound in several respects. In all of them the glucuronic acid amide is replaced by aminoglucose, i.e. the analogues contain a $-CH_2OH$ group on the 6′ carbon instead of a carboxamide $-CONH_2$ grouping. In compounds II and III the sarcosyl-D-seryl amide residue is bound to the 3′-carbon of glucose, whereas in compound IV the sarcosyl-D-seryl residue is bound to the 4′-carbon.

Replacement of the $-CONH_2$ group of gougerotin by a $-CH_2OH$ group in the analogue IV decreases the inhibitory activity approximately 10 times. This may indicate that the carboxamide group increases the affinity of the compounds in question to the active

Table 1
The effect of gougerotin and analogue (IV) on the CACCA–(AcLeu) and CACCA–Phe binding to ribosomes.

	Concn. M	CACCA–(AcLeu) binding		CACCA–Phe binding	
		cpm	%	cpm	%
Control	–	345	100	2680	100
Gougerotin	10^{-4}	535	155	2168	81
	10^{-3}	565	164	1256	47
Analogue (IV)	10^{-3}	357	104	2567	96
	2×10^{-3}	385	112	2243	80

Assay of CACCA–(AcLeu) binding was determined according to Celma et al. [4]. The incubation mixture containing CACCA–(Ac-^{14}C-Leu) (2200 cpm, specific activity 83 mCi/mmole) and 70 S ribosomes (390 μg protein) was incubated at 0° 40 min.

Assay of CACCA–Phe binding was determined according to Pestka [10]. The reaction mixture containing CACCA–^{3}H-Phe (10700 cpm, specific activity 21 Ci/mmole), 70 S ribosomes (170 μg protein) and 20% ethanol (v/v) incubated at 24° for 20 min.

The amount of bound substrate was calculated by difference of parallel incubations with and without ribosomes.

site of peptidyl transferase, though the grouping itself is not directly involved in the inhibitory mechanism. The position of the sarcosyl-D-seryl amide residue on the glucose moiety appears to be more important. Peptidyl transferase was inhibited only by compounds which had this residue attached on the 4′-carbon of glucose or its derivative (compounds I and IV). Derivatives with the sarcosyl-D-seryl amide chain attached to the 3′-carbon did not show any inhibitory effect on peptidyl transferase.

It has been proved recently [2, 7, 9] that the active site of peptidyl transferase is composed of two binding sites, the donor and the acceptor site, which specifically interact with the donor and acceptor substrate respectively. At concentrations which inhibit the transfer of an acylaminoacyl group, gougerotin and compound IV increase the amount of donor substrate bound to the donor site and decrease the amount of acceptor substrate at the acceptor site. For this reason we suggest that gougerotin and its derivative inhibit peptidyl transferase by competing with the acceptor substrate at the acceptor site. This view is in accordance with the fact that the structure of the two compounds is similar to that of 2′(3′)-*O*-aminoacyl cytidine, which is a good acceptor substrate in the reaction catalyzed by peptidyl transferase [7]. The increased binding of the donor substrate to the donor site may be caused by a conformation change at the donor site which occurs when the acceptor site has been occupied by the acceptor substrate.

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