CATALYSIS OF PEPTIDYL TRANSFER BY HUMAN TONSIL RIBOSOMES AND EFFECTS OF SOME ANTIBIOTICS

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1. Introduction

The peptide bond-forming reaction of protein synthesis in bacteria [1–3], yeast [4], protozoa [5], rat liver [6], and human tonsils [4] is catalyzed by a peptidyl transferase catalytic centre on the larger ribosomal subunit. The functioning of this centre can be specifically studied, uncoupled from other reactions of protein synthesis, by means of the "fragment reaction" (fig. 1). This reaction system has been fairly extensively characterized with *E. coli* ribosomes [1,3,8–10], but corresponding studies with eukaryote ribosomes have only just begun [4–6]. In the present paper we report some general characteristics of the fragment reaction using human tonsil ribosomes. Responses to monovalent and divalent cations and to alcohol are very similar to those with *E. coli* and yeast ribosomes, but there are some striking differences in sensivity to antibiotic inhibitors.

2. Methods

Ribosomes from human tonsils and rat liver were prepared as described elsewhere [11]. Subunits from the tonsil ribosomes were prepared by the method of Martin and Wool [12]. The standard incubation mixture for assay of the fragment reaction contained (before addition of alcohol) the following components: 0.05 M-Tris/HCl buffer (pH 7.4), 0.4 M KCl, 0.02 M-Mg acetate, 1 mM-puromycin, 1 mg/ml tonsil ribosomes, and CACCA-(^{3}H) leu-Ac (about 8 nM; specific

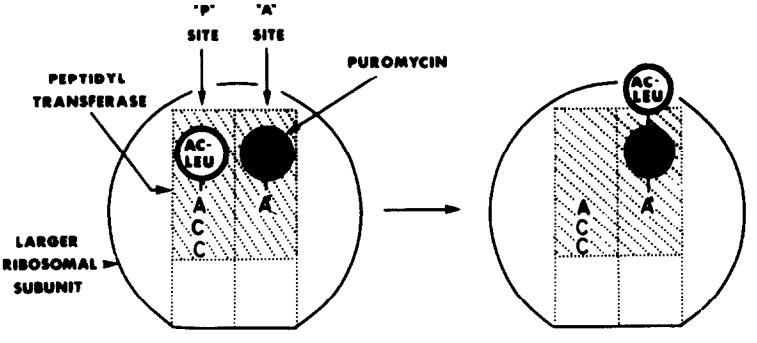

Fig. 1. Diagrammatic representation of the "fragment reaction" [4,7]. Acetyl-leucine is transferred from CCA to puromycin giving Ac-leu-puromycin and CCA. Other substrates can also be used [8]. The reaction is catalysed by preparations of the larger ribosomal subunit (as shown) or of complete ribosomes. The fragment reaction has the advantage that interactions between substrates and ribosome are confined to the immediate vicinity of the peptidyl transferase centre. Properties of the catalytic centre can thus be specifically examined.

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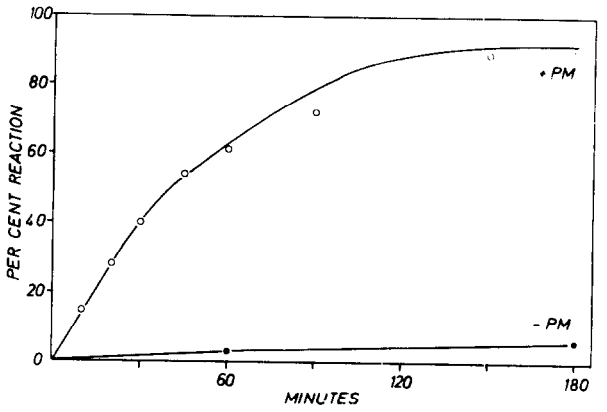

Fig. 2. Time course of reaction between CACCA-leu-Ac and puromycin. Conditions as in text.

activity about 22 curie/mmole; prepared as in [8]). 100 μl portions were equilibriated at 0°, and the reaction initiated by addition of 50 μl precooled ethanol. Formation of Ac-leu-puromycin was estimated by extraction with ethyl acetate ([9] modified from [13]). The extent of reaction is expressed as the percentage of CACCA-leu-Ac converted to Ac-leu-puromycin. Zero time blanks (less than 3% of added radioactivity) were substracted from all estimates.

3. Results

3.1. *Time course*

Fig. 2 shows that the reaction of CACCA-leu-Ac with puromycin was about 50% completed after 40 min incubation at 0°, and about 90% completed after 120 min. The initial rate of reaction was about twice that with rat liver ribosomes (table 1), about the same as with yeast ribosomes [4], and about 20% that obtained with the best preparations of *E. coli* ribosomes [14] at comparable molar concentration. In the absence of puromycin there was very little formation of ethyl acetate-soluble material.

3.2. *Requirements*

Table 1 shows that tonsil ribosomes can be replaced by their 60S but not their 40S subunits, and table 2 shows that the reaction is unaffected by fusidic acid, an inhibitor of transfer factor factor-2 [15–16], or by GMP-PCP (guanylyl methylenediphosphonate), a GTP antagonist [17]. These observations are in accord with the proposition [4] that peptide bond formation in mammals, as in lower species, takes place by a simple peptidyl transfer reaction, catalysed by an active centre on the larger ribosomal subunit.

The fragment reaction with tonsil ribosomes (table 1), as with ribosomes from *E. coli* [3,8,18] and yeast [4], is dependent upon K^+, Mg^{2+} and ethanol and takes place much less rapidly when CACCA-leu-Ac is replaced

Table 1
Factors affecting reaction of CACCA-leu-Ac with puromycin.

Experimental conditions	% fragment reacted
Expt. 1	
Standard system	40
minus ribosomes	0
tonsil ribosomes replaced by rat liver ribosomes	20
ribosomes replaced by 60S subunits	38
ribosomes replaced by 40S subunits	0
minus K^+	0
minus Mg^{2+}	0
minus ethanol	0
Expt. 2	
Standard system	44
CACCA-leu-Ac replaced by CACCA-leu	0

Conditions as in text unless otherwise indicated. Incubation was for 30 min at 0° C.

by CACCA-leu as peptidyl donor. The responses of the reaction with tonsil ribosomes to concentrations of K^+ (fig. 3) and Mg^{2+} (fig. 4) are similar to the responses with ribosomes from *E. coli* [9] and yeast (unpublished results).

3.3. *Effects of antibiotics*

Table 2 compares the effects of various antibiotic inhibitors of protein synthesis on the fragment reaction with ribosomes from human tonsils, yeast and *E. coli*. Chloramphenicol, lincomycin and spiramycin III inhibit the reaction with *E. coli* ribosomes but are inactive with yeast or tonsil ribosomes. In contrast, anisomycin (and tenuazonic acid to a lesser extent) inhibits the reaction with yeast and tonsil ribosomes but not with *E. coli* ribosomes. Sparsomycin, amicetin and gougerotin inhibit the reaction with all three types of ribosome. Anisomycin and gougerotin are more active against ribosomes from yeast than from human tonsils. These responses correspond with the known specificities of these antibiotics towards protein synthesis in different species [19,20: review].

The antibiotic cycloheximide, a protein synthesis inhibitor specific to eukaryote systems, has no effect on the fragment reaction with tonsil ribosomes (table 2) and little or no effect with yeast ribosomes. We conclude that this antibiotic acts on some component of the protein synthetic machinery other than the peptidyl transferase centre. The weak activity of tenuazonic acid against the fragment reaction with tonsil and yeast ribosomes leaves doubt as to its site of action.

4. Discussion

The peptidyl transfer reaction of protein synthesis has the following common characteristics in *E. coli*, yeast and man:

(a) the reaction is catalyzed by a centre on the larger ribosomal subunit;

(b) the peptidyl transferase centre is normally dormant in absence of mRNA and the smaller ribosomal subunit, but the activity can be expressed with isolated preparations of the larger subunit if alcohol is present;

Table 2
Effects of antibiotics on the fragment reaction with *E. coli*, yeast and tonsil ribosomes

Antibiotic	Reaction with tonsil ribosomes (as % of control) in presence of 1mM antibiotic	Concentration (μM) of antibiotic giving 50% inhibition with ribosomes from:		
		E. coli	yeast	tonsils
Chloramphenicol	*c.* 100	20	inactive	inactive
Lincomycin	*c.* 100	3	inactive	inactive
Spiramycin III	*c.* 100	15	inactive	inactive
Anisomycin	15	inactive	1	10
Tenuazonic acid	54	inactive	>1000	1000
Sparsomycin	0	50	5	10
Amicetin	30	40	1000	100–1000
Gougerotin	15	30	10	100
Cycloheximide	*c.* 100	inactive	>1000	inactive

GMP-PCP and fusidic acid were without effect in all three systems. Data have been compiled from several different assays. Except for assays with *E. coli* ribosomes, conditions were as in text, and incubations were for 30 min at 0° C. Concentration of inhibitor giving 50% inhibition is the mean value of two assays. The lack of effect of GMP-PCP, fusidic acid, anisomycin, cycloheximide and tenuazonic acid on the fragment reaction with *E. coli* ribosomes was observed using similar conditions, but other data for *E. coli* are from another publication [10], in which CAACCA-met-f was employed as substrate. Data for yeast (*Saccharomyces cerevisiae*) are from [4]. Further references to the antibiotics and their sources are noted in a recent review [20].

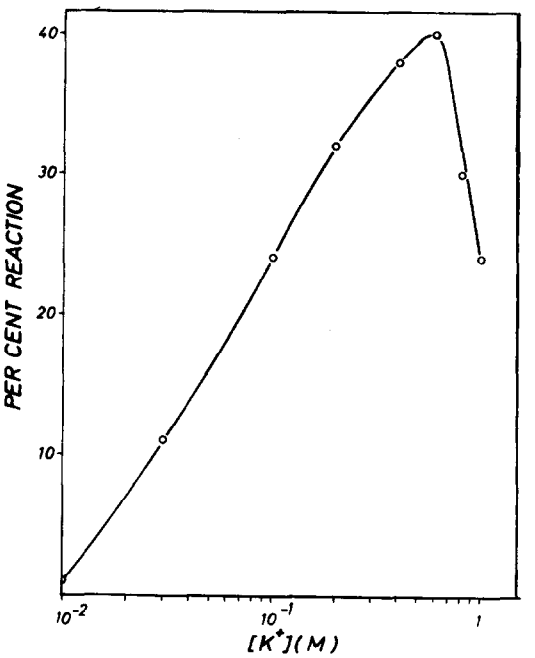

Fig. 3. Effect of K^+ concentration on reaction of CACCA-leu-Ac with puromycin. Conditions as in text, except that K^+ concentration was varied as indicated. Incubation was for 30 min at 0° C.

(c) the reaction requires monovalent and divalent cations, and has characteristic responses to their concentrations;

(d) the reaction is inhibited by sparsomycin, amicetin and gougerotin.

In spite of these similarities in the peptidyl transfer reaction with different species, there must be certain differences in the fine structures of the peptidyl transferase centres of prokaryote and eukaryote ribosomes in view of the specificites of chloramphenicol, lincomycin and spiramycin III to ribosomes from bacteria [20], and of anisomycin to ribosomes from yeast, protozoa (G.A.M.Cross, personal communication), rat liver [6] and human tonsils. The potent activity of amisomycin is of particular interest, since it is the only eukaryote-specific inhibitor of protein synthesis at present known to act on the peptidyl transferase centre. The fragment reaction might provide a useful assay for further screening and development of peptidyl transferase-specific agents which discriminate between different species or, perhaps, between different cell types (including tumour) of the same species.

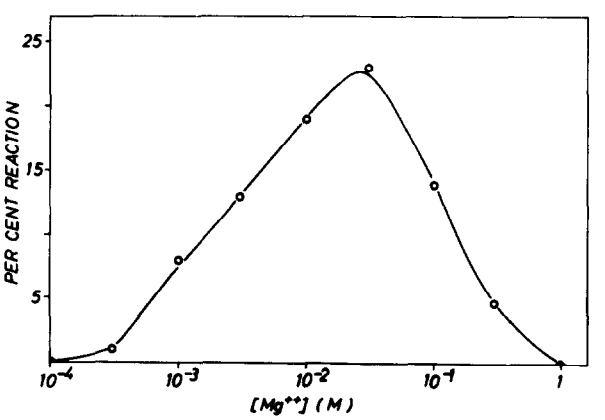

Fig. 4. Effect of Mg^{2+} concentration on reaction of CACCA-leu-Ac with puromycin. Conditions as in text except that Mg^{2+} concentration was varied as indicated. Ribosomes were pre-dialysed for 2 hr against 0.1 mM Mg acetate, 50 mM tris-acetate buffer (pH 7.4), 10 mM NH_4 acetate. Incubation was for 30 min at 0°C.

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