SELECTIVE INHIBITION OF RIBOSOMAL RNA SYNTHESIS IN SACCHAROMYCES CARLSBERGENSIS BY 5-FLUOROURACIL

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In bacteria, the pyrimidine analogue 5-fluorouracil (FU) is known to be incorporated into RNA and to exert severe effects on protein biosynthesis. Thus in E. coli the analogue causes an alteration of several newly synthesized proteins, e.g. β-galactosidase (Bussard et al., 1961; Nakada et al., 1964). This effect has been explained to be the result of the incorporation of the analogue into messenger RNA leading to a miscoding of the amino acid sequence (Champe et al., 1962).

Another effect of 5-fluorouracil is a strong inhibition of the formation of ribosomes (Aronssen, 1961; Kono et al., 1964), yet the cells continue to produce a high molecular weight RNA with sedimentation properties and a base composition resembling that of normal ribosomal RNA when the values of uracil and 5-fluorouracil are taken together (Andoh et al., 1965; Kono et al., 1964).

The effect of 5-fluorouracil on RNA and protein synthesis in yeast has been studied thusfar only by Kempner (1961) using Candida utilis. In the present paper the effect of the analogue on RNA synthesis in cells and protoplasts of Saccharomyces carlsbergensis has been investigated. The results show that, contrary to the results obtained in studies on bacteria, 5-fluorouracil causes the synthesis of ribosomal RNA to be strongly inhibited, whereas the formation of messenger and soluble RNA is allowed to continue.

MATERIALS AND METHODS

5-Fluorouracil was obtained either as a generous gift from Dr. Alexander Tomàsz of the Rockefeller Institute, New York, or purchased from Serva (Heidelberg).

S. carlsbergensis was grown and cells were converted to protoplasts as described in a previous paper (de Kloet, 1961). The preparation and sucrose density gradient centrifugation as well as the estimation of the base composition of newly synthesized RNA with the aid of ^{32}P-labeled orthophosphate have been described elsewhere (de Kloet, 1965, 1966). The separation of the ribosomal subunits by sucrose density gradient centrifugation was carried out according to Henshaw (1964). For the preparation of ribosomes, protoplasts were lysed in 2 ml of a solution containing per ml: 10 µmoles of $MgCl_2$, 20 µmoles of Tris-HCl buffer pH 7.6, 50 µmoles of KCl and 5 mg of Na-deoxycholate. Ribosomes were obtained as a crude fraction by 2 hours centrifugation at 100,000 g. Unless stated otherwise, the medium used for the incorporation of the radioactive precursors contained per ml: 2 mg of casamino acids (Difco); 5 mg of glucose; 20 µmoles of potassium phosphate buffer pH 6.2; 2 µmoles of $MgCl_2$ and 20 µg of unlabeled adenine. 120 mg of mannitol was added where protoplasts were used. Protoplasts and cells were suspended in the medium to give an optical density of 0.5 to 1 at 500 mµ. The incorporation of ^{14}C-leucine into protein and ^{14}C-adenine into RNA was measured after the usual washing procedures with hot or cold trichloroacetic acid etc.

RESULTS

When protoplasts or cells of S. carlsbergensis are incubated in a medium containing 5-fluorouracil, RNA formation is strongly inhibited, whereas protein synthesis is impaired to a much lesser degree (Fig. 1). However when the incorporation of ^{14}C-leucine into the proteins of ribosomal subunits was studied it was found that the analogue interfered severely with the formation of these subunits (Fig. 2), an observation similar to that made earlier for bacteria (Aronsson, 1961; Kono et al., 1964).

To study the effect of the analogue on ribosome synthesis somewhat more closely, the base composition as well as the se-

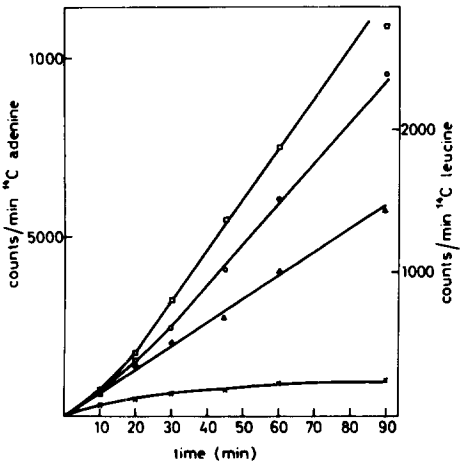

Fig. 1. The effect of 5-fluorouracil on protein and RNA synthesis in S. carlsbergensis. Yeast cells (E at 500 mμ = 0.5) were incubated at 30°C in 25 ml of the medium described in the text, with or without 5-fluorouracil (200 μg/ml). At zero time 75 μg 8-^{14}C-adenine (spec. act. 10.0 mC/mmole) or 50 μg 1-^{14}C-leucine (spec. act. 36.6 mC/mmole) was added. At the times indicated 1.5 ml samples were withdrawn and pipetted into 1.5 ml cold 10 per cent trichloroacetic acid. Incorporation of ^{14}C-leucine into protein and ^{14}C-adenine into RNA was measured as usual. Values are expressed as total counts per min per sample.
o — o = ^{14}C-leucine; Δ — Δ = ^{14}C-leucine + 5-fluorouracil
□ — □ = ^{14}C-adenine; x — x = ^{14}C-adenine + 5-fluorouracil

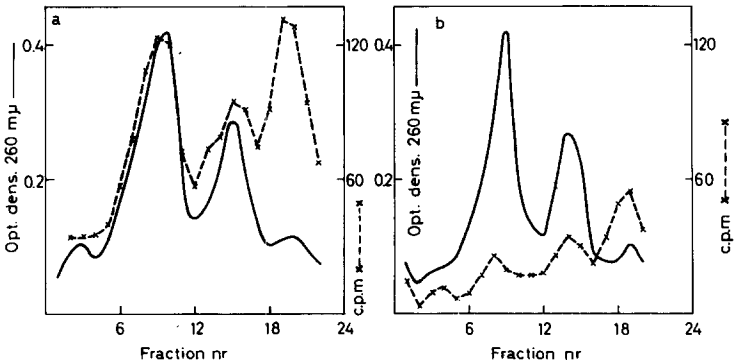

Fig. 2. The effect of 5-fluorouracil on the formation of ribosomal subunits. Yeast protoplasts (E at 500 mμ = 1) were incubated for 75 min at 30°C in 4 ml of a medium as described under "METHODS" with 10 μg 1-^{14}C-leucine (spec. act. 36.6 mC/mmole) and with or without 200 μg 5-fluorouracil per ml. Protoplasts were collected by centrifugation and the ribosomes isolated and separated into their subunits as described before. The optical density of the fractions was measured after six fold dilution; the hot trichloroacetic acid insoluble radioactivity was measured after the addition of 2 mg yeast protoplast protein to the fractions as a carrier.
a) = without 5-fluorouracil; b) = with 5-fluorouracil

dimentation properties of the RNA synthesized under the influence of 5-fluorouracil were investigated.

Fig. 3 shows that the RNA formed in the presence of the analogue sediments as heavy material with its main peak of activity sedimenting in front of the stable ribosomal RNA species, together with RNA of low molecular weight. The pattern resembles that of the pulse labeled RNA and the RNA formed in the presence of cycloheximide, described earlier (de Kloet, 1965, 1966). When ^{14}C-labeled 5-fluorouracil was used instead of ^{14}C-labeled adenine, essentially similar patterns were obtained, demonstrating that the analogue is readily incorporated into both high and low molecular RNA. The data in Fig. 3 show

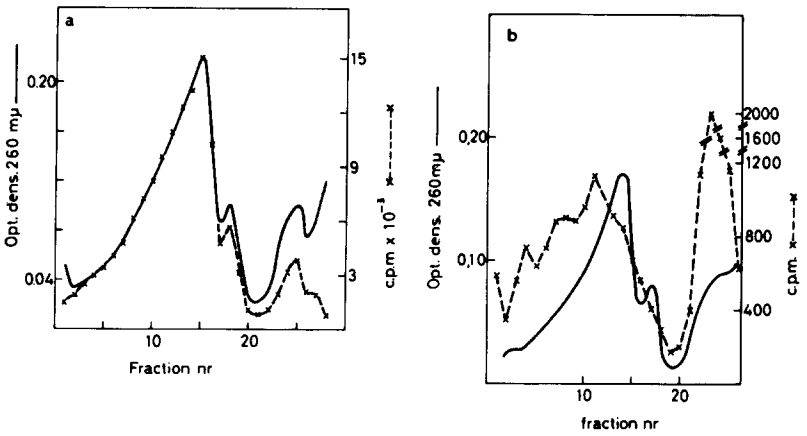

Fig. 3. The effect of 5-fluorouracil on the sedimentation pattern of newly synthesized yeast RNA. Protoplasts (E at 500 mμ = 1) were incubated for 60 min at 30°C in 25 ml of a medium as described in the text, with or without 5-fluorouracil (200 μg/ml). At zero time 75 μg of 8-^{14}C-adenine (spec. act. 10.0 mC/mmole) was added. RNA was isolated and sucrose gradient analysis was carried out as described before. The optical density of the fractions was measured after thirty fold dilution; the cold trichloroacetic acid soluble radioactivity was measured after the addition of 2 mg carrier yeast protoplast protein. a) = without 5-fluorouracil; b) = with 5-fluorouracil

furthermore that the formation of high molecular weight RNA is much more inhibited by 5-fluorouracil than the synthesis of the low molecular weight RNA.

Table I shows the results of the base composition analyses of the RNA formed in the presence of the analogue. In the Dowex-formate system employed in these studies fluorouridylic

acid is eluted just after the uridylic acid peak (Horowitz et al., 1959). The fractions containing ^{32}P-labeled fluorouridylic acid were detected routinely by monitoring the eluate from the column. In agreement with the results of studies on other organisms (Horowitz et al., 1959) it was found furthermore that the radioactivity of 2-^{14}C-5-fluorouracil appears only as ^{14}C-fluorouridylic acid, hence also yeast is apparently unable to convert 5-fluorouracil into fluorocytosine.

Table I. The effect of 5-fluorouracil on the base composition of newly synthesized yeast RNA.

	C	A	G	U(T)	FU
yeast DNA	18	32	18	32	
yeast ribosomal RNA	19.3	26.3	27.7	26.6	
HMW RNA without FU	19.4	26.2	27.6	26.6	
HMW RNA with FU (200 µg/ml)	18.9	29.9	22.7	7.7	20.8
yeast soluble RNA	28.5	21.6	27.9	22.0	
LMW RNA without FU	27.0	22.6	28.0	22.4	
LMW RNA with FU (200 µg/ml)	26.6	23.0	26.8	7.2	16.4

Protoplasts were incubated for 90 min as described under Fig. 3, except for the reduction of the phosphate concentration to 0.2 µmole/ml and the addition of 0.3 mC carrier free ^{32}P-labeled orthophosphate. High molecular weight (HMW) RNA (>12S) and low molecular weight (LMW) RNA (<12S) were separated by sucrose density gradient centrifugation, precipitated with alcohol and the base composition of the newly synthesized RNA was estimated as described earlier (de Kloet, 1966). Values for the base composition of yeast ribosomal RNA and yeast soluble RNA were obtained as described elsewhere (de Kloet, 1966). The yeast DNA base composition was obtained from the litterature (Zamenhof et al., 1950). Values are expressed as moles per cent.

The data in Table I show in the first place that in the presence of 5-fluorouracil a large proportion (up to 75 per cent) of the uridylic acid in the newly synthesized RNA is replaced by fluorouridylic acid. Furthermore the results show that when the values of uridylic acid and fluorouridylic acid are taken together, the low molecular weight RNA has a high G-C content typical for soluble RNA, whereas the base composition of high molecular weight RNA is distorted to that of yeast DNA, a finding similar to that obtained in earlier studies on the effect of cycloheximide on RNA formation in yeast (de Kloet, 1965, 1966).

DISCUSSION

The results presented in this study show that in S. carlsbergensis 5-fluorouracil causes a severe inhibition of ribosome and ribosomal RNA formation, whereas the synthesis of soluble RNA and of a high molecular weight RNA with a DNA like base composition, most likely messenger RNA, are allowed to continue to a considerable extent. These results differ from those obtained in studies on E. coli where apparently "normal" ribosomal RNA containing 5-fluorouracil is formed, although the experiments presented here do not exclude the synthesis of a small amount of ribosomal RNA or a high molecular weight precursor thereof. The reason for this selective inhibitory effect on ribosomal RNA synthesis in yeast is not clear and a direct effect of the analogue on the enzymatic system involved in the formation of ribosomal RNA or an extreme instability of a newly synthesized ribosomal RNA containing 5-fluorouracil cannot be excluded. However since a similar phenomenon has been observed with cycloheximide, an indirect action mechanism common to both compounds might be possible. 5-Fluorouracil and cycloheximide are both known to interfere in some way with protein synthesis and hence the possibility exists that a continuous formation of a special protein is needed for the continuous synthesis of ribosomal RNA (cf. Kurland et al., 1962). On the other hand both compounds have been found to inhibit the formation of DNA (Kerridge, 1958; Cohen et al., 1958) and hence the observed inhibitory action on ribosomal RNA synthesis might be caused by this interference with DNA formation.

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