STIMULATION OF SYNTHESIS OF MACROMOLECULES IN FOLATE-STARVED *STREPTOCOCCUS FAECIUM* BY FOLINIC ACID

Seppo S. SARIMO

Department of Biochemistry, University of Turku, 20500 Turku 50, Finland

Received 30 October 1972

1. Introduction

It has been shown previously that *Streptococcus faecium* (S. faecalis) can grow in a folate-free medium if supplemented with serine, methionine, and purines and pyrimidines [1, 2]. In those defined conditions of culture formylation of methionyl-$tRNA_f^{Met}$ is not required for the initiation of protein synthesis [3, 4]. Although folate-dependent and -independent initiations in *S. faecium* are readily interconvertible without significant adaptation requirements, the folate-deficiency is characterized by somewhat slower growth-rate [3, 4]. This might be a consequence of the reduced capability of unformylated initiator to form an initiation complex [5, 6].

The present report describes the changes in the patterns of synthesis of protein, RNA, and DNA during the growth-rate transition when folate-starved, actively growing cells are transferred to a medium containing 5-formyltetrahydrofolate (folinic acid, citrovorum factor, CF). As a result of this transfer the rate of RNA synthesis speeds up almost immediately, and is followed by an increase in the rates of protein and DNA synthesis as well as of growth. That burst of RNA synthesis does not take place if protein synthesis is inhibited by chloramphenicol (CAL).

2. Materials and methods

Streptococcus faecium (ATCC 8043) [7] was grown without aeration in folate-free medium as described earlier [5]. The inoculum was prepared from cells transferred several times to this medium. Actively growing cells were collected by centrifugation and suspended in the original volume of folate-deficient medium. An inoculum to give a cell density of 5–10 Klett-units was used. Growth at 37° was followed by measuring the turbidity of culture (Klett colorimeter, filter 62). When CF (leucovorin from American Cyanamid Company) was added its final conc. was 1 μg/ml. To inhibit protein synthesis CAL, 100 μg/ml, was used.

Protein, RNA, and DNA synthesis were determined by using cultures labeled, respectively, with 0.15 μCi/ml of [^{14}C]leucine (327 mCi/mmole), 0.1 μCi/ml of [^{14}C]uracil (60 mCi/mmole), and 10 μCi/ml of [^{3}H]thymine (120 Ci/mmole). In all experiments an excess of unlabeled precursors were present in the following concentrations: about 5 mM leucine, 0.18 mM uracil, and 0.4 mM thymine. All the radioactive material was purchased from New England Nuclear.

At various times, 0.25 ml culture samples were added to 2.5 ml of ice-cold 5% trichloroacetic acid (TCA). Tubes containing precipitates were kept in ice for at least 0.5 hr, poured on Whatman glass-fiber filters (GF/A) and washed with ice-cold 5% TCA and acetone. Hot acid hydrolysis (90–95° for 20 min) was included in the treatment of leucine-labeled samples. All the radioactive samples were counted with a liquid scintillation counter (Wallac, Decem NTL^{314}) in a toluene-based scintillation solution (5 g PPO and 100 mg POPOP in 1 ℓ of toluene). To compare the cultures supplemented with CF to the control cultures without CF, the incorporation data are also expressed as relative values with respect to control.

3. Results and discussion

When CF is added into a folate-free *S. faecium* culture, the growth rate is increased so that the doubling time is about 25% shorter than when CF (or folate) is omitted [3, 4] (fig. 1). In order to accelerate their growth after a transfer to a supplemented media, cells must increase the rate of synthesis of their macromolecules: DNA, RNA, and proteins. This can be seen in fig. 1. The sequence of events during this transition is more clearly illustrated in fig. 2 where the incorporation data are given as relative values. In general terms the pattern of the stimulation of macromolecule synthesis and growth is in agreement with the earlier discoveries of "shift-up" (transfer) phenomenon [8].

Since the synthesis of 10-formyltetrahydrofolate: methionyl-tRNA transformylase is not dependent on folate [4], and since the low molecular weight products of folate metabolism are always provided, the metabolic effect of CF-supplementation should be confined to polypeptide initiation. The results of the earlier *in vitro* binding studies of formylated and unformylated methionyl-$tRNA_f^{Met}$ also suggest that unformylation of the initiator is accompanied by the reduced rate of protein synthesis [5, 6].

The foremost stimulation of RNA synthesis by CF is in good accord with the fact that the rate of protein

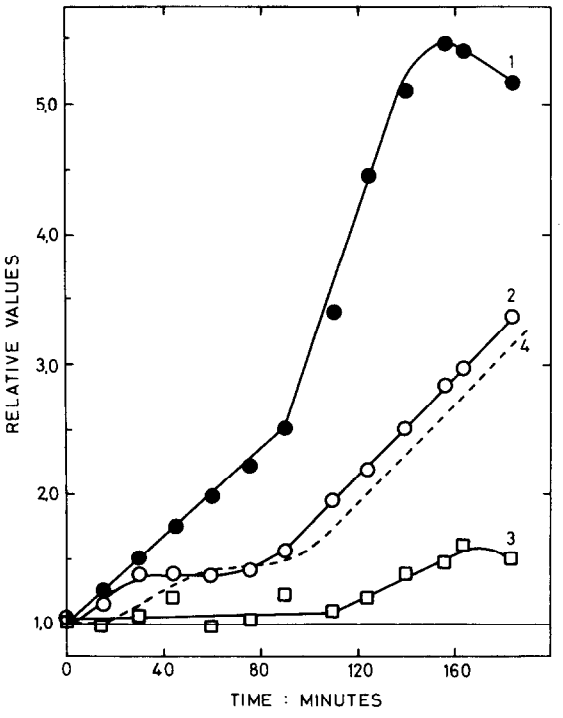

Fig. 2. Relative values of the rates of RNA (1), protein (2) and DNA (3) synthesis, and of growth (4) after supplementation of folate-starved cultures of *S. faecium* with CF.

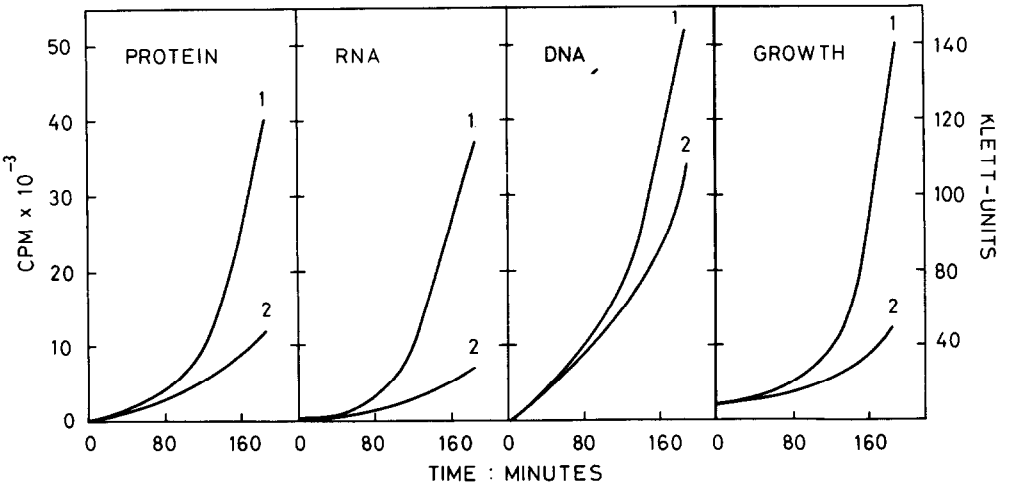

Fig. 1. Growth and synthesis of protein, RNA and DNA in the cultures of *S. faecium* with (1) and without (2) CF. The incorporation of radioactive precursors into protein and DNA are given as cpm per 6.5 ml (total vol. of culture) and into RNA as cpm per 0.25 ml (vol. of samples removed).

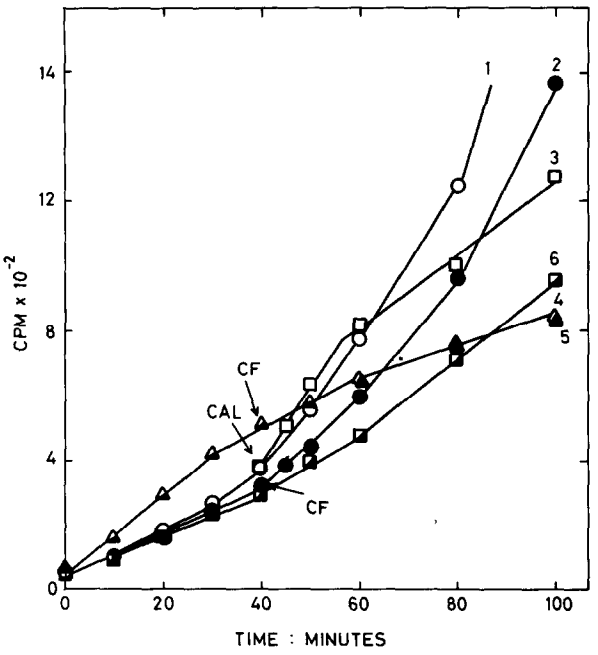

Fig. 3. Effect of CF and CAL on the rate of RNA synthesis in folate-starved cultures of *S. faecium*. Incorporation of [^{14}C]-uracil is given as cpm per 0.25 ml of culture. (1) With CF added at 0 min; (2) with CF added at 40 min; (3) with CF added at 0 min and CAL added at 40 min; (4) with CAL added at 0 min; (5) with CAL added at 0 min and CF added at 40 min; (6) control, no CF or CAL.

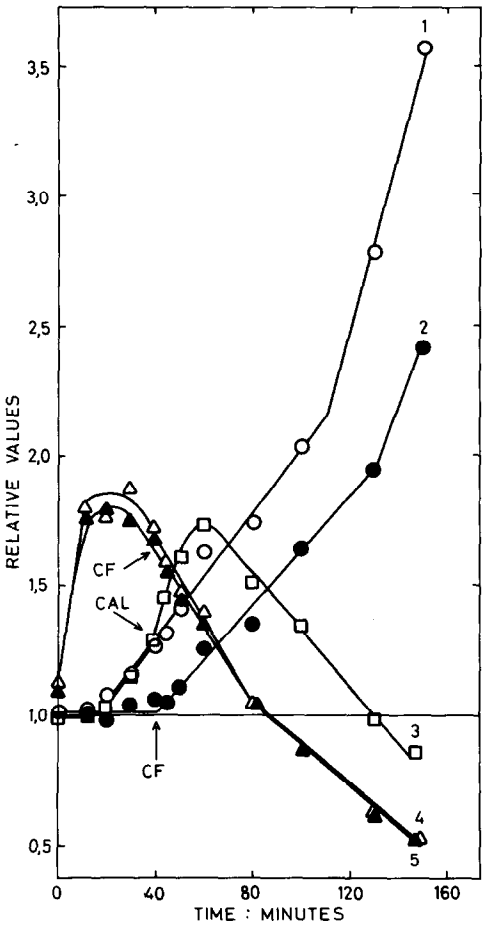

Fig. 4. Relative values of the rate of RNA synthesis after addition of CF and/or CAL into folate-starved cultures of *S. faecium* For the description of lines see the legend of fig. 3.

synthesis is proportional to the cellular RNA content. It cannot be increased until the number of ribosomes becomes larger. Thus the above results suggest that the apparently instant formation of *N*-formylmethionyl-tRNA$_f^{Met}$ after addition of CF provides *S. faecium* cells primarily with a mechanism for a more effective protein production which initially appears as increased rate of RNA synthesis.

To test the feasible ability of CF to stimulate RNA synthesis independently from protein synthesis, CAL was used to stop protein synthesis. Figs. 3 and 4 show that no stimulation of RNA synthesis by CF takes place if protein synthesis is inhibited. On the other hand there is a burst of RNA production immediately after addition of CAL. This is related to CAL's ability to uncouple RNA biosynthesis from the control of protein biosynthesis [9]. Normally the two processes are closely related, as also shown in *S. faecium* (table 1), except in a relaxed strain of *E. coli* in which intensive RNA synthesis continues although protein synthesis is arrested by an amino acid deficiency [10].

We can conclude from these results and discussion that the syntheses of RNA, protein, and DNA in *S. faecium* are related sequentially when folate-starved cells are supplemented with CF. Although the increase in RNA synthesis is most distinct it does not take place if protein synthesis is blocked. Thus the primary target of CF must be the stimulation of protein synthesis through the formation of formylated initiator. However, the change in protein and DNA synthesis as

Table 1
The effect of amino acid starvation and CAL on the biosynthesis of proteins and RNA in the folate-free cultures of *S. faecium*.

Culture	cpm incorporated		KU*	% incorporated	
	[^{14}C]Leu	[^{14}C]uracil		Leu	Uracil
Growing	2730	17,393	60	100	100
Growing + CAL	117	12,244	40	4	71
Starving	178	277	8	6.5	1.6
Starving + CAL	78	3,578	5	3	21

* The increase in Klett-units during pulse-labeling of 30 min.

Cells grown without folate were washed with tryptophane-deficient medium, divided into two portions, and suspended into the same medium with (try+) or without (try−) tryptophane. 0.5 ml aliquots of try+ culture were pulsed immediately with [^{14}C]leucine and [^{14}C]uracil for 30 min. Starved, try−, cultures were pulsed similarly after no growth could be registered. Final conc. of CAL was 100 μg/ml.

well as in growth to the new, faster rate does not occur until a certain cellular level of RNA has been attained.

Acknowledgements

This research project was financially supported by a grant from the Cultural Fund of Finland (Suomen Kultuurirahasto).

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