THE FORMATION OF BACTERIOPHAGE T7 AND T3 LYSOZYMES FROM INACTIVE PRECURSORS

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1. Introduction

The early genes of the closely related bacteriophages T3 and T7 are transcribed by the host RNA polymerase whereas the late functions are under the control of a phage dependent polymerase, an early enzyme [1,2]. However, the regulation of these different transcriptional processes seems to be leaky, since, for example, it was shown that T7 lysozyme is synthesized in vivo as a late and in vitro as an early function [3].

Hagen and Young [3] reported that there is a lag of about 3–4 min between the appearance of T7 lysozyme mRNA and the appearance of active enzyme during T7 development at 30°C. They further showed that this result was due neither to an inhibition of lysozyme activity in early extracts nor to the maturation of the enzyme from an inactive precursor. Here we report data suggesting that, at least in vitro, the T7 and T3 lysozymes are first produced in an inactive form.

2. Materials and methods

The preparation of protein fractions and ribosomes and the assay conditions for the synthesis and the activity of the enzymes were as described previously [4,5]. Lysozymes (EC 3.2.1.17) of T3 and T7 bacteriophages were assayed by a modification [4] of the method of Leutgeb and Schwarz as described by Schweiger and Gold [6]. Besides lysozyme, the RNA polymerases (EC 2.7.7.6) of T3 and T7 and the *S*-adenosyl-methionine cleaving enzyme (EC 3.3.1.–) of T3 were studied. All assays for the synthesis of protein or enzymes were performed in the presence of 3 mM spermidine. The level of protein synthesis was determined by following the incorporation of radioactive amino acids into an acid insoluble form; samples were precipitated with 5% trichloroacetic acid, followed by incubation for 20 min at 95°C [7], filtration through glass fibre filters GF/A (Whatman, England), drying and counting [4]. In all experiments no background values (except the counter background) have been subtracted.

3. Results and discussion

Activity of an enzyme can be observed only after transcription of the relevant gene, translation of the mRNA, folding and possible modification of the polypeptide chain. In the case of T7 or T3 lysozyme the time required for these processes was about 7 min at 30°C. (fig.1). In principle one can determine the time required for a possible modification: If an enzymatically inactive polypeptide chain is converted to an active form in a process requiring some measurable time, one should find a further increase in enzymatic activity after translation of the message has been blocked. Under these circumstances the polypeptide chains which would become active enzymes are those whose synthesis has already been completed. By determination of the lag phase for the appearance of enzymatic activity, either before or after further incubation in the presence of inhibitors of translation, it should be possible to distinguish between the time required for the synthesis of the polypeptide chain and that required for the synthesis of active enzyme.

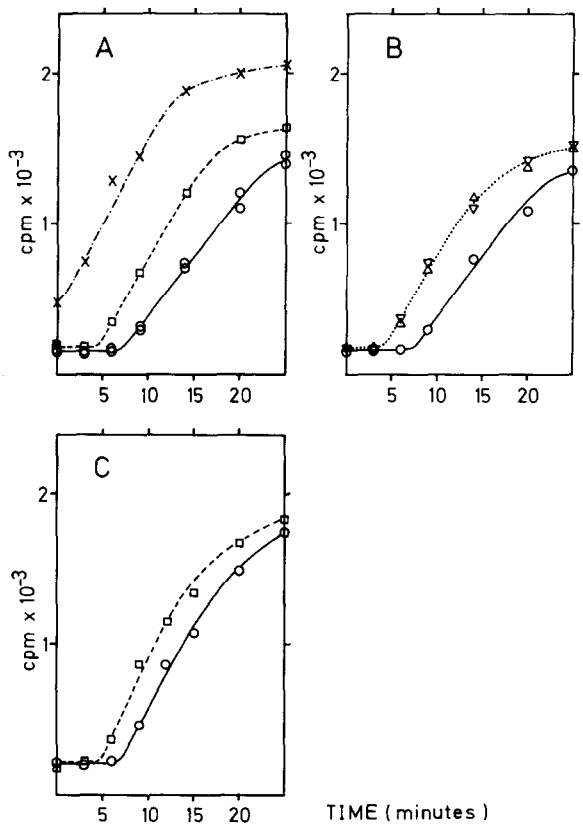

Fig.1. Synthesis of lysozyme at 30°C in a coupled in vitro system from *E. coli* [4]. (A) and (B) show the time course of T7 lysozyme synthesis under the conditions described in Materials and methods except that in (A) 8 mM Mg^{2+} and 10 mM phospho-*enol*-pyruvate and in (B) 11 mM Mg^{2+} and 20 mM phospho-*enol*-pyruvate were added to the synthesis mixture; (C) shows the time course of T3 lysozyme synthesis determined as above but in the presence of 13 mM Mg^{2+} and 28 mM phospho-*enol*-pyruvate. Experiments A, B and C were each carried out with different preparations of protein fraction, of ribosomes and of the filters used for the lysozyme assay; this helps to avoid artefacts due to peculiarities of the materials used [4]. The results however cannot be compared with respect to the yield of lysozyme synthesized. At each time point protein synthesis was blocked by the addition of antibiotics as follows: (v. . . . . .v) 0.6 mM chloramphenicol (△ . . . . . △) 0.1 mM puromycin, (□ . . . . . □) 0.6 mM chloramphenicol + 0.1 mM puromycin, in each case incubation was continued for a further 10 minutes at 30°C prior to assay. (○ . . . . . ○) is the 'normal' time course of lysozyme synthesis; enzyme assays were performed immediately without further incubation. (X – . – . – X) is the time course of lysozyme synthesis after addition at each specified time of an equal volume of fresh unincubated synthesis mixture; synthesis was continued for a further 10 min at 30°C prior to assay.

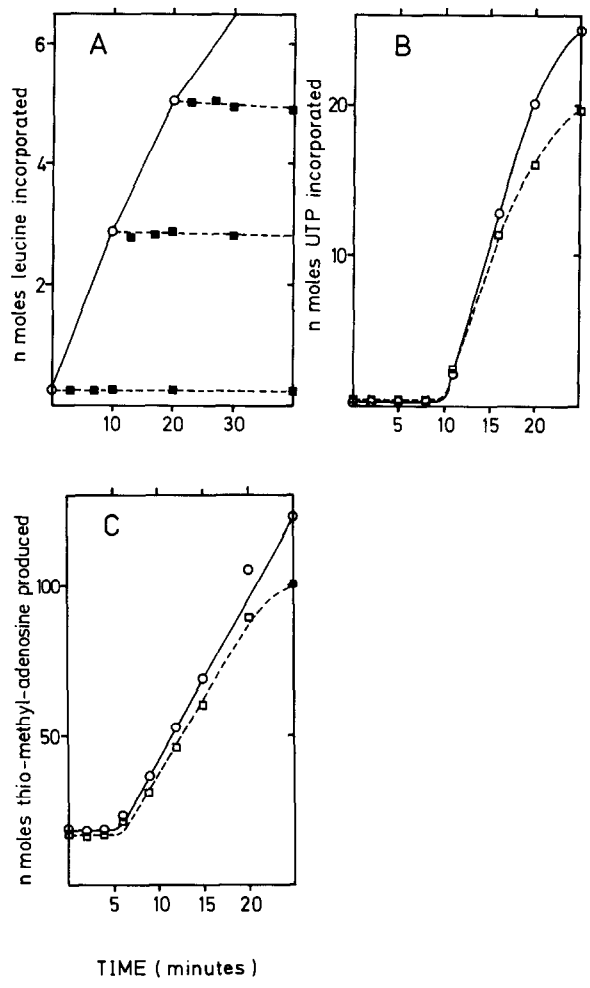

Fig.2. Synthesis of total protein and enzymes at 30°C in a coupled in vitro system from *E. coli* (A) shows the time course of total protein synthesis under the conditions described in Materials and methods but in the presence of 8 mM Mg^{2+} and 10 mM phospho-*enol*-pyruvate; at 0, 10 and 20 min 0.6 mM chloramphenicol + 0.1 mM puromycin were added. (B) shows the time course of T7 RNA polymerase synthesis in the presence of 8 mM Mg^{2+} and 10 mM phospho-*enol*-pyruvate under the conditions as above. (C) shows the time course of the synthesis of T3 *S*-adenosyl-L-methionine cleaving enzyme determined as above but in the presence of 6 mM Mg^{2+} and 6 mM phospho-*enol*-pyruvate. (○———○) is the 'normal' time course of protein or enzyme synthesis. (□– – – –□) At each time point protein synthesis was blocked by the addition of 0.6 mM chloramphenicol + 0.1 mM puromycin and the incubation was continued for a further 10 min at 30°C prior to assay of the enzyme. (■– – – –■) shows the synthesis of total protein in the presence of the antibiotics.

The difference between these two lag phases would give the time required for activation.

As is shown in fig.1, a difference in the lag phases of about 3 min was obtained for the synthesis of lysozyme in such experiments. This result was the same under different assay conditions ranging from 6 mM Mg^{2+} and 6 mM phospho-*enol*-pyruvate (not shown here) to 13 mM Mg^{2+} and 28 mM phospho-*enol*-pyruvate for the syntheses of both T3 and T7 lysozyme. There was no difference whether either chloramphenicol or puromycin or both drugs together were added (fig.1A and 1B). Since only puromycin should release nascent polypeptide chains from the ribosome (see ref. [8]), it seems to be unlikely that the effect is due to release of incompleted but active protein, Comparable results were obtained at 37°C where the lag phase was reduced from about 6 to 3 min.

The possibility that an unstable inhibitor was responsible for this effect was ruled out by a further experiment; addition of fresh assay mixture to an actively synthesizing in vitro system at different times did not significantly influence the synthesis of active enzyme (fig.1A). This result is in agreement with the data reported by Hagen and Young [3].

The described effect on the synthesis of lysozyme was not due to a continuation of protein synthesis in the presence of the antibiotics as is shown in fig.2. Total protein synthesis came to an immediate stop after addition of the drugs (fig.2A). No difference in the length of the lag phases was observed when the syntheses of T7 RNA polymerase (fig.2B) or T3 *S*-adenosyl-L-methionine cleaving enzyme (fig.2C) were followed with or without added inhibitors of protein synthesis in the same way as described above. The results of these control experiments were the same under the variety of assay conditions as given above for lysozyme.

These experiments strongly suggest that T7 and T3 lysozymes are converted from an inactive nascent form to the active enzyme within 3 min under the given conditions. The discrepancy between the in vitro data reported here and the findings of Hagen and Young [3] which were obtained in vivo cannot be explained at present. This might perhaps be due to the fact that the lysozymes are synthesized in vitro as early proteins and therefore an activation process could be more clearly seen. Experiments are in progress to characterize an enzyme which would be responsible for the described effect.

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