INDUCED SYNTHESIS OF AN ENVELOPE PROTEIN BY THYMINE STARVATION OF *E. COLI* B/r

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1. Introduction

We have reported elsewhere [1] the discovery of an envelope protein synthesised in a periodic fashion in synchronous cultures of *E. coli* B/r. This protein has an apparent mol. wt. of 76 000 as determined by polyacrylamide gel electrophoresis in SDS (SDS-PAGE). The normal time of synthesis of this protein appears to coincide with the termination of rounds of DNA replication in synchronous cultures, growing in a medium which supports a mean generation time of 65 min. In this report we describe experiments, involving inhibition of DNA synthesis by removal of thymine from the growth medium, performed with exponentially growing cultures of *E. coli* B/r, which indicate that the synthesis of an apparently identical protein is preferentially induced by thymine deprivation.

2. Methods

The bacterial strain and the growth conditions will be described in detail elsewhere. Briefly, the strain was *E. coli* B/r LEB 16 F$^-$*lac*Z, *thy*A, *drm* obtained from Meacock and Pritchard [2]. Bacterial cultures were pulse labelled at intervals with [^{14}C] leucine (Amersham: 311 mCi/mmol) by removal of 1.0 ml of culture into 1.0 ml of fresh, prewarmed medium, containing 1 μCi of isotope. After 4 min, incorporation of isotope was stopped by the addition of chloramphenicol (final concentration 300 μg/ml) and leucine (final concentration 2 mg/ml). Envelope preparation, polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS-PAGE) and estimation of radioactivity from autoradiograms were essentially as described previously [3,4]. Removal of thymine from the exponentially growing culture was achieved by filtration of *E. coli* B/r through a membrane filter (Millipore grade HA 0.45 μm pore size) followed by washing and resuspension in twice the volume of prewarmed, fresh medium, without thymine

3. Results and discussion

Fig.1 shows an autoradiogram of some SDS-PAGE profiles of envelope samples obtained from (1) a thymine starved culture, (2) a synchronous culture, pulse labelled at a time prior to the synthesis of the periodic protein and (3) a synchronous culture labelled at the end of the division cycle which shows the labelled periodic protein. The sample from the thymine starved culture is considerably overexposed, however it is apparent from this slab gel that there is a prominent band in this profile at a position which corresponds exactly to that of the 76 000 dalton periodic protein. On this basis we conclude that the polypeptide preferentially labelled in the thymine starved culture is probably identical to the periodic protein.

In order to examine the kinetics of synthesis of the 76 000 dalton protein, a culture of *E. coli* B/r was pulse labelled at different times before and after thymine starvation, and the rate of total protein synthesis and the rate of synthesis of the 76 000 dalton protein was determined. Pulse-chase experiments have confirmed that such experiments directly measure the rate of synthesis, rather than the rate of synthesis *and* concomitant insertion into the

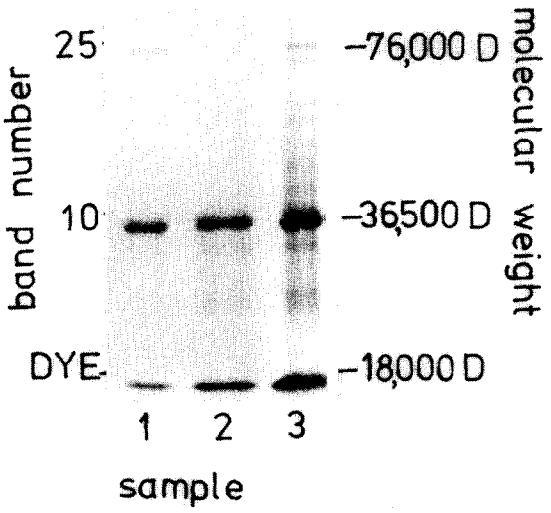

Fig.1. Autoradiograph of 12.5% polyacrylamide-SDS gel profiles of envelopes prepared from (1) an exponentially growing culture of *E. coli* B/r pulse labelled thirty minutes after removal of thymine, (2) bacteria from a synchronous culture pulse labelled at birth, and (3) a synchronous culture pulse labelled 40 min after birth to label the periodic protein.

envelope of *E. coli* B/r [4]. The autoradiographic gel profile of envelope samples obtained from this experiment is shown in fig.2. During growth in the presence of thymine the amount of label in the 76 000 band remains a constant fraction of total envelope protein. In contrast, the major observable effect of thymine starvation, confirmed by microdensitometer scans, is to increase the relative amount of radioactivity being incorporated into the 76 000 dalton band over a period of 40 min. Since the degree of blackening of the X-ray film in

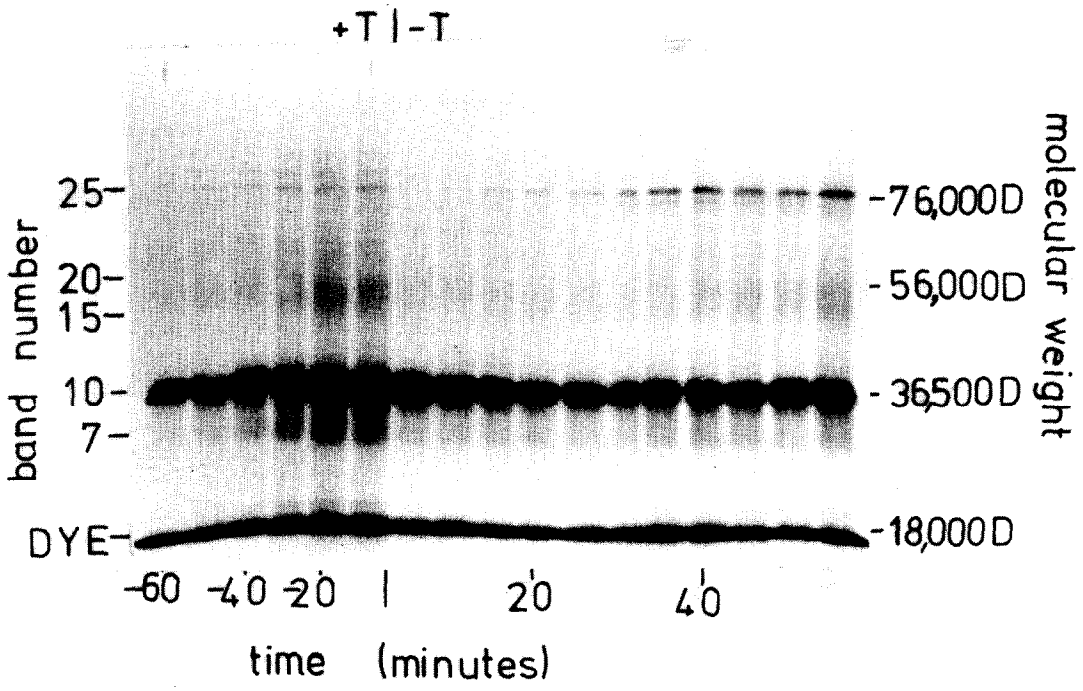

Fig.2. Autoradiograph of 12.5% polyacrylamide-SDS gel profiles of envelopes prepared from an exponentially growing culture of *E. coli* B/r before and after thymine starvation. Samples were pulse labelled for four minutes with 1 μCi [^{14}C]leucine (311 μCi/mmol) and then envelopes were prepared and subjected to electrophoresis.

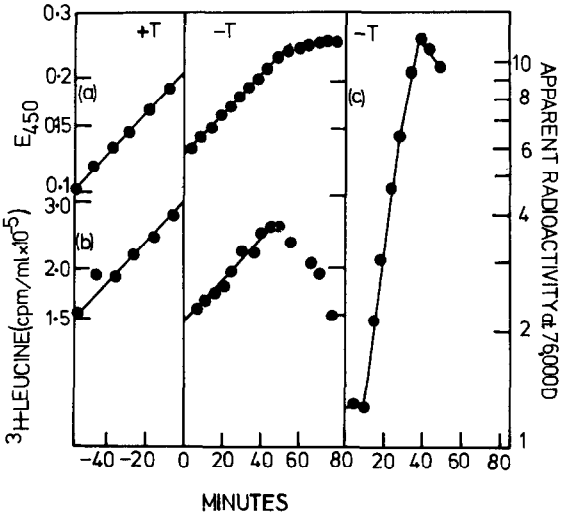

Fig.3. Rate of synthesis of total protein and 76 000 dalton protein. An exponentially growing culture was deprived of thymine at time zero. At intervals, samples were pulse labelled with [^{14}C]leucine for 4 min, envelopes were prepared and in addition an aliquot of the clarified sonicate was removed from corresponding samples to determine the incorporation of leucine into total protein. The envelopes were run on a 12.5% acrylamide gel and the relative incorporation of radioactivity into the 76 000 dalton band determined by densitometry of the resulting autoradiogram shown in fig.2. (a) Optical density of culture; (b) rate of total protein synthesis; (c) relative rate of synthesis of the 76 000 protein.

individual bands over most of the range used in this study [4], is proportional to the amount of radioactivity in that band, the rate of synthesis of the 76 000 dalton protein, both absolutely and relative to that of other envelope proteins, is dramatically increased. Thus the synthesis of this protein may be said to be induced.

The kinetics of induction of the 76 000 dalton protein are shown more clearly in fig.3 where the rate of synthesis of the band material, as calculated from the area beneath the autoradiogram peak from each sample, is compared with the rate of total cellular protein synthesis following thymine starvation.

The results presented in this paper show that the synthesis of a specific envelope protein is induced during inhibition of DNA replication by thymine starvation. This is not an unknown phenomenon and in particular the work of Pardee and co-workers [5] has shown that several proteins, in particular an inner membrane protein of mol. wt. 40 000 (protein X) behave in a similar fashion. However, the 76 000 dalton protein, which is probably an outer membrane protein (A. Boyd, personal communication) is unusual in that it is apparently synthesised in a periodic fashion in synchronously growing cultures of *E. coli* B/r. Moreover, it is apparently only synthesised at a time which corresponds to the termination of rounds of DNA replication at the particular growth rate used in these experiments. It is therefore a possibility that this protein is only made when DNA synthesis stops.

A surprising feature of the experiment described here is that the appearance of protein X is not observed, despite the fact that this protein apparently constitutes a large fraction of the envelope protein synthesised in *E. coli* B/r when DNA synthesis is blocked with nalidixic acid [5]. The reason for this absence of protein X is not clear but at least two possibilities exist. Firstly, it is known that there are significant differences between isolates of *E. coli* B/r used in different laboratories [6], and the absence of protein X from the *E. coli* B/r strain used here may be due to such a difference. Secondly, it has been shown that mutants displaying tolerance to colicin E2 (Cet$^-$) synthesise, under normal growth conditions, an envelope protein [3] with a molecular weight close to that of protein X. It is therefore possible that *cet* represents a regulatory locus for the synthesis of protein X and that Cet$^-$ mutants make this protein constitutively. The strain used here, in addition to its properties listed above is in fact colicin E2 tolerant and it is possible that this strain carries a mutation which rather than leading to constitutive production of X, prevents any synthesis of protein X even after inhibition of DNA synthesis. Experiments to test these ideas are currently being undertaken.

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