A Theoretical Analysis of the Kinetics of Bacterial Exoprotein Formation following Inhibition of Transcription

By GEOFFREY COLEMAN

Department of Biochemistry, University Hospital & Medical School, Clifton Boulevard, Nottingham NG7 2UH

(Received 31 October 1977)

INTRODUCTION

During a study of the characteristics of extracellular enzyme secretion by Bacillus amyloliquefaciens in the presence of inhibitors of transcription (Both et al., 1972; Gould, May & Elliott, 1973), data were obtained which were interpreted in terms of an accumulation of a vast excess of unstable exoenzyme-specific mRNA within the bacterial cells (Glenn et al., 1973). However, this interpretation was not supported by the independent work of Brown & Coleman (1975a, b) and Coleman & Brown (1975) who measured exoenzyme mRNA throughout the growth cycle by DNA : RNA hybridization.

This communication represents an attempt to clarify the position concerning the existence, or otherwise, of an accumulation of exoprotein mRNA. For this purpose an accumulation of mRNA will be defined as any excessive mRNA produced which might be passively present in cells, which is not part of active polysomes but which will, nevertheless, replace active polysome-attached mRNA as the latter is depleted. The term 'mRNA pool' has not been used in order to avoid possible ambiguities relating to its precise meaning.

RESULTS AND DISCUSSION

The consequences of inhibiting transcription on the kinetics of exoprotein formation in situations in which different components of the translatory and secretory apparatus are limiting have been considered.

1. Exoprotein mRNA is limiting and is wholly associated with actively translating polysomes.

This is the simplest case to consider. Inhibiting transcription of exoprotein genes would be accompanied by an exponential decline in polysome-associated exoprotein mRNA. This would be reflected in a decline in exoprotein formation (Fig. 1, curve a).

The kinetics of this process would be the same irrespective of whether one considers that exoprotein synthesis is initiated on free polyribosomes within the bacterial cytoplasm and that the specific secretory determinant resides in the N-terminal amino acid sequence of the exoprotein itself, as suggested by Milstein et al. (1972), or, alternatively, that exoprotein mRNA associated with ribosomes is bound at special 'translation-extrusion sites' located on the cell membrane, as proposed by Both et al. (1972), provided that the availability of these sites imposes no limitation.

2. 'Translation-extrusion sites' are limiting with an accompanying accumulation of exoprotein mRNA. This embraces the model proposed by Both et al. (1972) which can exist in two possible forms: one in which the excess of free mRNA 'queuing up' for translation is considered to be stable and the other in which it is considered to be unstable. However, in both cases, on inhibiting transcription, exoprotein would continue to be formed linearly, at its maximum rate, as long as all the 'translation-extrusion sites' remain saturated i.e. until the exoprotein mRNA has decayed to the level at which 'translation-extrusion sites' cease to be limiting. From this point onwards the capacity for exoprotein formation would
Fig. 1. A comparative analysis of the progress of bacterial exoprotein formation in the absence of transcription (a) when exoprotein mRNA is limiting and is wholly associated with actively translating polysomes, (b) when 'translation-extrusion sites' are limiting with an accompanying accumulation of exoprotein mRNA, and (c) when a component of the protein-synthesizing machinery other than mRNA is limiting and an accumulation of exoprotein mRNA but not cell protein mRNA exists.

decrease with a half-life determined by the decay of its mRNA (Brown & Coleman, 1975b).
The characteristics of exoprotein synthesis expected from this model are shown in Fig. 1 (curve b). The two distinct phases would be present irrespective of whether the excess of free mRNA is considered to be stable or unstable. However, in the former situation the initial linear formation of exoprotein, immediately after inhibition of transcription, would be extended reflecting its ability to prolong the period over which it might be used to 'top up' actively translating polysomes.

3. A component of the protein-synthesizing machinery other than messenger RNA is limiting. It is convenient to envisage the inhibition of transcription in a system in which availability of ribosomes is limiting translation and in which there exists an accumulation of mRNA for both cellular and extracellular proteins or either of the two.

If there is an accumulation of both cellular and extracellular protein mRNAs then, as the active mRNA of polysomes decays, 'topping up' with excess mRNA would maintain the level of active polysomal species. This would apply, irrespective of whether the excess mRNA is stable or unstable in its passive form, until the accumulation is depleted, at which stage ribosomes would cease to be limiting. This model is similar to that described in (2) above and the kinetics of exoprotein formation would follow curve b (Fig. 1) provided the relative proportion of exoprotein to cell protein polysomes does not change.

When an accumulation of cell protein mRNA exists over and above that which is polysome-bound and all the exoprotein mRNA is associated with polysomes active in translation, then a decline in exoprotein formation would be observed in an inhibited system with the kinetics shown in curve a (Fig. 1). Concomitant with mRNA decay, ribosomes released from both exoprotein-producing and cell protein-producing polysomes would combine with the excess cell protein mRNA to produce an increasing level of cell protein polysomes, as long as an excess of mRNA remains available, resulting in an increasing production of cell proteins.

A variant of this situation would be observed under conditions in which exoprotein mRNA alone is in excess. In this case loss of exoprotein mRNA by decay in active polysomes would be restored from the accumulated excess. Further, as cell protein mRNA decays in active polysomes, additional ribosomes would be made available which also would
combine with the accumulated exoprotein mRNA. The production of exoprotein under these circumstances would follow curve c (Fig. 1) and the kinetics would be the same whether or not an involvement of ‘translation-extrusion sites’ (Both et al., 1972) is postulated, provided they are not and do not become limiting.

An important conclusion to be drawn from Fig. 1 is that, whichever component of the system is limiting, the formation of exoprotein would continue for a significant period after inhibition of transcription. The shortest period of synthesis following inhibition would occur when exoprotein mRNA is limiting such that exoprotein formation first approaches a plateau value after 5 half-lives of mRNA decay (curve a). In the other two cases (curves b and c), in both of which there is considered to be an accumulation of exoprotein mRNA, synthesis would continue for longer than 5 half-life periods. However, the practical applicability of this fact as a basis for distinguishing between the different models is limited by the accuracy with which exoprotein or specific exoenzymes can be measured. Even with the most reliable data it would not be easy to determine the precise point at which a plateau is reached. Therefore, its usefulness will be limited to recognition of a very large accumulation of mRNA, sufficient to permit several half-life periods of synthesis of exoprotein prior to becoming limiting.

A more discriminating test may seem to be provided by a carefully controlled study of the characteristics of exoprotein secretion immediately following inhibition of transcription. In the absence of an accumulation of mRNA, the rate of exoprotein formation would be rapidly reduced during the first half-life period (curve a). By contrast, in the presence of an accumulation of mRNA, exoprotein would continue to be formed at the same (curve b) or at a higher rate (curve c) after inhibition than before until exoprotein-specific mRNA became limiting.

Considering the results of Gould et al. (1973), Glenn et al. (1973), Semets et al. (1973), Stinson & Merrick (1974) and Boethling (1975) in relation to the present analysis leads to the conclusion that there are insufficient kinetic data available in the literature to substantiate the claim of an accumulation of exoprotein-specific mRNA in bacterial cells.

REFERENCES


