Short Communications

Protein Synthesis in the Presence of Non-Ionic Detergents in a Cell-Free System from Rabbit Reticulocytes

By S. OLSNES, U. SPAEREN, R. HEIBERG and A. PIHL

Norsk Hydro's Institute for Cancer Research, Montebello, Oslo 3, Norway

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Detergents are often used to release membrane-bound polyribosomes in postmitochondrial supernatants (Blobel & Potter, 1967; Olsnes, 1971) or to extract polyribosomes and other cytoplasmic components from non-homogenized cells (Perry & Kelley, 1968; Gielkins *et al.*, 1971). When protein-synthesizing systems are prepared the detergents are usually removed, and the effect of different detergents on protein synthesis as such does not appear to have been studied. Frequently it would be advantageous to be able to study protein synthesis directly without having to centrifuge down and resuspend the polyribosomes. We now report that in concentrations up to 1% the non-ionic detergents Triton X-100 and Brij-58 do not interfere with protein synthesis in a cell-free system.

Results and discussion

The rabbit reticulocyte lysate system described by Lingrel (1972) was used. This system is known to possess good re-initiation properties, a fact allowing studies, not only of peptide chain elongation, but also of the sensitive mechanisms of peptide chain initiation. Moreover, the polyribosomes in a reticulocyte lysate are not bound to membranes (Bulova & Burka, 1972), a fact that permits the study of detergents on protein synthesis as such without any additional effect due to release of the polyribosomes from membranes.

The results given in Fig. 1 demonstrate that in the absence of detergents the incorporation of [14 C]-leucine into protein occurs at a linear rate for 20–40 min at 28°C. When aurintricarboxylic acid in a final concentration of 0.1 mM was added, the incorporation proceeded linearly only for 5–10 min. Since aurintricarboxylic acid in this concentration inhibits re-initiation of new peptide chains, but does not inhibit the completion of already initiated chains (Grollman & Stewart, 1968; Hoerz & McCarty, 1971), the present results indicate that under our conditions 'run-off' was completed in about 10 min and that in the absence of aurintricarboxylic acid re-initiation did indeed take place.

When 1% (w/v) of Triton X-100 or Brij-58 was added to the system the incorporation of amino acids occurred at the same rate as in the control. Thus in concentrations up to 1% these non-ionic detergents

apparently inhibit neither the elongation nor the initiation in this system. However, when a 5% (w/v) concentration of the detergents was used the incorporation was considerably decreased (results not

Fig. 1. Effect of different detergents on protein synthesis in a cell-free system from rabbit reticulocytes

The system was prepared as described by Olsnes & Pihl (1972) and divided into six parts. One was used as control (●); others were made up to contain 1% sodium deoxycholate (▲), 1% Triton X-100 (×), 1% deoxycholate + 1% Triton X-100 (■), 1% Brij-58 (△) and 0.1 mM-aurintricarboxylic acid (○). The incubations were carried out at 28°C. Samples (25 μ l) were taken at the times indicated, and the incorporation of [14 C]leucine was determined by measuring the acid-precipitable radioactivity as described by Olsnes & Pihl (1972).

Fig. 2. Translation of added mouse globin mRNA in a lysate from rabbit reticulocytes in the absence and in the presence of Triton X-100

A rabbit reticulocyte lysate was prepared for protein synthesis *in vitro* (Lingrel, 1972) in two parallel samples. One sample (a) was used as control, the other one (b) was made up to contain 1% Triton X-100. Each sample was then divided into two parts. To one part [^{14}C]leucine was added, and to the other part [^3H]leucine and $12\mu\text{g}$ of mouse globin mRNA were added. After incubation at 28°C for 2h the samples with and without added mRNA were combined, and the proteins were separated on a column of CM-cellulose (Whatman CM-52) (Lingrel, 1972). E_{280} ($\bullet$), ^{14}C radioactivity ($\blacktriangle$) and ^3H radioactivity ($\times$) were determined in each fraction as described by Spaeren *et al.* (1972).

shown). In contrast, addition of 1% (w/v) of the anionic detergent sodium deoxycholate completely abolished the incorporation of [^{14}C]leucine (Fig. 1). Interestingly, the simultaneous presence of Triton X-100 provided partial protection against the inhibitory effect of deoxycholate. It is likely that the inhibition of protein synthesis by deoxycholate can to a large extent be accounted for by release of ribosomes from the mRNA. Thus we have previously shown (Olsnes *et al.*, 1972) that at this temperature (28°C) incubation of polyribosomes with deoxycholate causes a complete release of monoribosomes from the intact mRNA, an effect that was prevented when Triton X-100 was present as well.

To study with a more sensitive technique the effect of non-ionic detergents on peptide chain initiation,

the translation of added purified mouse globin mRNA was tested by the procedure described by Lingrel (1972). To one of the samples was added purified mouse globin mRNA whereas no mRNA was added to the control. As labels [^3H]leucine and [^{14}C]leucine respectively were used. After incubation the samples were mixed, unlabelled mouse haemoglobin was added as a marker and the globin chains were separated by chromatography on a column of CM-cellulose (Whatman CM-52). Under these conditions the mouse β -globin chains are clearly separated from the mouse α -chains and the rabbit α - and β -chains, which are all eluted together in a single peak. Hence in this system synthesis of mouse β -chains will be revealed as a peak of ^3H radioactivity at the location of the added mouse

β -chain marker, as shown in Fig. 2(a), where mouse globin mRNA had been added. When 1% of Triton X-100 (Fig. 2b) or Brij-58 (results not shown) was present in the system similar results were obtained, demonstrating that these detergents did not interfere with peptide chain initiation.

Even though non-ionic detergents have recently been included in several biological systems, such as antigen-antibody reactions (Crumpton & Parkhouse, 1972; Stavnezer & Huang, 1971; Rhoads *et al.*, 1971) and enzyme studies (Bowers *et al.*, 1967), it was nevertheless somewhat unexpected that the complicated machinery of protein synthesis may function in an apparently normal fashion in the presence of 1% Triton X-100 or Brij-58. The present results may have several practical applications. For example, it will now be possible to study the role of membranes in protein synthesis by measuring amino acid incorporation in a cell-free system before and after dissolving the endoplasmic reticulum by a non-ionic detergent.

Materials

L-[4-³H]Leucine (specific radioactivity 22 Ci/mmol) and L-[U-¹⁴C]leucine (specific radioactivity 344 Ci/mol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Triton X-100 and aurointricarboxylic acid were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. Sodium deoxycholate (special enzyme grade) was obtained from Mann Research Laboratories, New York,

N.Y., U.S.A. Brij-58 was obtained from Atlas Chemical Industries Inc. Chemicals Division, Wilmington, Del., U.S.A. CM-cellulose (Whatman CM-52) was obtained from W. and R. Balston Ltd., Maidstone, Kent, U.K.

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