SECRETION OF PROTEINS FROM LIVER CELLS IS SUPPRESSED BY THE PROTEINASE INHIBITOR *N*-α-TOSYL-L-LYSYL CHLOROMETHANE, BUT NOT BY TUNICAMYCIN, AN INHIBITOR OF GLYCOSYLATION

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1. Introduction

The biochemical events involved in the regulation of protein secretion in the liver have not been fully elucidated. It is possible that post-translational modifications of secretory proteins, such as specific proteolytic cleavage and attachment of carbohydrate residues, play a role in regulating protein secretion. However, of proteins synthesized in the liver, only albumin and transferrin have been shown to undergo proteolytic modification prior to secretion [1,2]. The attachment of carbohydrate to transferrin is not essential for secretion [3].

We have compared the importance of proteolysis and glycosylation for the secretion of protein from liver cell suspensions. Addition of *N*-α-tosyl-L-lysyl chloromethane, a proteinase inhibitor, led to a decrease of the secretion of all proteins studied whereas tunicamycin, an inhibitor of protein glycosylation, had no effect on secretion.

2. Materials and methods

Tunicamycin was a gift from Dr Robert L. Hamill, E. Lilly and Co., Indianapolis. *N*-α-tosyl-L-lysyl chloromethane hydrochloride, was obtained from Calbiochem. Inc., San Diego, CA. L-[1-^{14}C]leucine, 59 Ci/mol, was from The Radiochemical Centre, Amersham.

Abbreviation: TLCK, *N*-α-tosyl-L-lysyl chloromethane

Cell suspensions were prepared and incubated as in [4] except that hyaluronidase was omitted from the perfusion medium. Cells were separated from medium by centrifugation for 45 s at 30 × *g*. The 30 × *g* supernatant was cleared of any remaining cells by centrifugation for 10 min at 1000 × *g*. The 1000 × *g* supernatant was dialyzed extensively against 10 mM Tris–HCl (pH 7.8). Radioactivity in total protein was determined on filter paper discs [5]. Radioactivity in albumin, transferrin or α_1-acid glycoprotein in samples of the dialyzed medium (1000 × *g* supernatant) was determined as follows: Carrier protein (either rat plasma or the respective protein isolated from rat plasma) and excess rabbit antiserum raised against the particular protein were added to the samples, mixed, incubated at 37°C for 1 h and then 4°C for 3 h, and precipitated protein was collected by centrifugation for 15 min at 1000 × *g*. The precipitates were washed 5–8-times with 10 mM Tris–HCl (pH 7.7) digested with a 1 M solution of *p*-(di-isobutyl-cresoxyethoxyethyl) dimethyl benzyl ammonium hydroxide in methanol and counted in a liquid scintillation cocktail [6]. The levels of ATP, ADP and AMP in the cells were determined enzymatically [7,8].

Transferrin was purified from rat plasma by fractionation with ammonium sulphate, column chromatography on DEAE-cellulose and Sephadex G-100, and polyacrylamide gel electrophoresis. α_1-Acid glycoprotein was purified from rat plasma by fractionation with ammonium sulphate/trichloroacetic acid, followed by column chromatography on Amberlite CG-50 and DEAE-cellulose.

3. Results

3.1. *Effect of TLCK on the secretion of proteins from liver cells in suspension*

The effect of TLCK on the secretion of protein from suspensions of liver cells is shown in fig.1. In the medium from the controls, radioactivity in total protein, albumin and transferrin continued to increase after termination of protein synthesis at the 30 min time point. In contrast, radioactivity in total protein, albumin and transferrin increased only slightly in the medium from TLCK-treated samples.

In order to check the viability of cells incubated in the presence of TLCK, its effect on the adenine nucleotide levels of the cells was investigated. The levels of ATP, ADP and AMP in cell suspensions were determined following incubation for 50 min with varying concentrations of TLCK. In the presence of 10–50 μg/ml TLCK, the level of ATP ranged from 75–93% of that found in cells incubated without TLCK, while the ADP and AMP values were from 56–105% and 74–130%, respectively. The lowest levels of ATP in the cells were sufficient to support protein synthesis at 88% and protein secretion at 93% compared to cells containing the highest levels of ATP (K. E., J. Urban, and G. S., unpublished results).

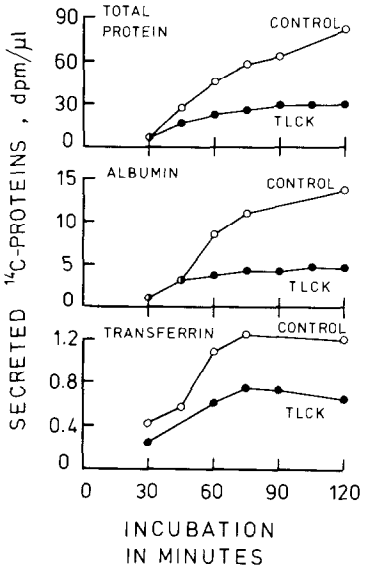

Fig.1. Effect of *N*-α-tosyl-L-lysyl chloromethane on the secretion of proteins from liver cells in suspension. Cells were incubated for 30 min at 37°C with 1 μCi/ml L-[^{14}C]-leucine, at which time 23 μM cycloheximide was added. The suspension was then divided into a control portion and a portion to which 500 μg/ml TLCK was added. Both portions were incubated at 37°C for a further 90 min. Samples of suspension were removed at 15 min intervals during the 90 min incubation. Cells and medium were separated and radioactivity in total protein, albumin and transferrin was determined in the medium from each sample as in section 2. (○—○) no TLCK; (●—●) 500 μg/ml TLCK.

3.2. *Effect of tunicamycin on secretion of proteins from liver cells in suspension*

As shown in table 1, the level of protein synthesis in cells incubated in the presence of tunicamycin was inhibited by 31–36% compared to cells incubated without tunicamycin. Similarly, the amount of radioactive protein secreted into the medium was reduced by 32–40% in the presence of tunicamycin. At all concentrations of tunicamycin studied, the ratio of both total [^{14}C]protein in the medium and of [^{14}C]-albumin in the medium to that of total [^{14}C]protein in the cells was close to unity, indicating that tunicamycin did not inhibit the secretion of either total protein or albumin from the cells in suspension. Also the values for [^{14}C]transferrin and ^{14}C-labelled α_1-acid glycoprotein in the medium showed little or no inhibition of the secretion of these proteins by tunicamycin. The levels of ^{14}C-labelled α_1-acid glycoprotein secreted were lowered only slightly at concentrations of tunicamycin (2 and 4 μg/ml) which were 8-fold higher than those inhibiting immunoglobulin secretion by plasmacytomas by >85% [9].

4. Discussion

The finding that TLCK, a proteinase inhibitor, suppresses the secretion of protein from liver cells in suspension leads to the question of its possible site(s) of action. Proteolysis may occur at several steps in the secretory pathway. The first site could be the cleavage of the 'pre'-segment from the N-terminus of newly synthesized proteins (reviewed in [1]). However, since the 'pre'-forms of proteins are seldom detected in tissue, it seems that their pool size is small and hence inhibition of removal of the 'pre'-segment could not by itself account for the magnitude

Table 1
Effect of tunicamycin on synthesis and secretion of proteins in suspensions of liver cells

Tunicamycin (μg/ml)	Cells	Medium			
	% Total [^{14}C]protein	% Total [^{14}C]protein	% Total [^{14}C]albumin	% Total [^{14}C]transferrin	% Total ^{14}C-labelled α_1-acid glycoprotein
0	100	100	100	100	100
0.25	69	68	71	84	61
0.50	68	62	66	78	50
1.0	65	60	60	81	60
2.0	65	60	63	n.d.	43
4.0	64	62	58	81	43

Liver cells in suspension were incubated for 90 min with 1 μCi/ml L-[^{14}C]leucine in the presence of the indicated doses of tunicamycin. Following termination of incubation, cells and media were separated and radioactivity in total protein, albumin, transferrin and α_1-acid glycoprotein was determined as in section 2. All determinations are the result of incubations with 2 samples of cell suspension

of the inhibition of secretion due to TLCK observed in this work. It therefore seems likely that TLCK exerts its effect at a stage in secretion after removal of the 'pre'-segment from proteins.

Inhibition by TLCK of the removal of the 'pro'-segment from pro-proteins would explain the observed inhibition of albumin secretion from cells incubated with TLCK. However, if this were the only site of action for TLCK, it would also imply the existence of a 'pro'-protein form of transferrin, the secretion of which was inhibited by TLCK (fig.1). However, it seems unlikely that a 'pro'-transferrin exists in rat liver [10].

During the secretory process, vesicles are constantly forming and fusing with larger membranes. If either the formation or fusion of vesicles requires proteolysis, this offers another possible site at which TLCK could be acting to inhibit protein secretion.

Tunicamycin is known to block the glycosylation of newly synthesized protein [9,11–14] by inhibiting the formation of *N*-acetylglucosamine-containing lipid intermediates involved in the assembly of the core regions of oligosaccharide chains linked N-glycosidically to protein [14–18]. Whilst it is not clear whether glycosylation is necessary for the secretion of all or only some specific immunoglobulins [9,19–20], tunicamycin is known to inhibit the intracellular migration of proteins of both Sindbis virus and vesicular stomatitis virus [21]. From the results reported here, it seems that glycosylation is not required for secretion of protein from rat liver cells in suspension, since incubation with tunicamycin had little effect on the secretion of either albumin, a carbohydrate-free protein, or the two glycoproteins, transferrin and α_1-acid glycoprotein. These results are in agreement with the lack of effect by tunicamycin on the secretion of transferrin or the apoprotein B chain of very low density lipoprotein from rat and chick hepatocyte monolayers, respectively [3]. It seems that the attachment of a completed carbohydrate moiety is not mandatory for the secretion of glycoproteins.

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