PROTEASE ACTIVITY ASSOCIATED WITH HELA CELL RIBOSOMES

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Summary

HeLa cells contain endoprotease activity, detected by a sensitive, solid phase assay. The endoprotease has the ability to cleave a variety of protein substrates and is trypsin-like in its sensitivity to inhibitors. The activity is in part associated with cellular ribosomes and polysomes. A variety of biological and physical-chemical treatments which alter ribosomes or protein synthesis also directly affect the ribosomal protease activity.

Protein cleavage has been observed in the biosynthesis of cellular and viral proteins, and proteolysis has been implicated in the regulation of various cell functions (1-4). It is of interest to characterize the cellular proteases involved in these reactions, however, little is known of their composition, specificity or intracellular location.

Problems arise in attempts to characterize the nature of the proteolytic activity. These include low activities in crude cell extracts, instability of the enzymatic activity, endogenous inhibitors, and unknown substrate requirements (5). Nevertheless, recent progress has been reported, and the proteases of interest are usually of the serine type (often trypsin-like) and have neutral pH optima (5-8). Previous reports indicated the presence

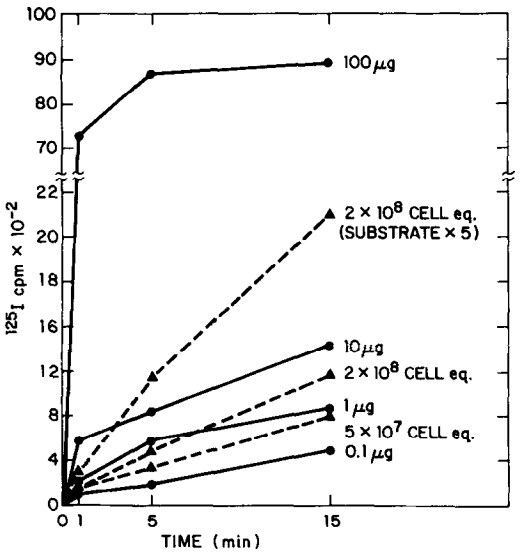

Figure 1. Assay of proteolytic activity of bovine trypsin (EC 3.4.4.4) and of HeLa cell extracts using substrate bound to sepharose beads. Bovine hemoglobin (one mg/ml) was reacted with ^{125}iodide according to Marchalonis (19). The labeled substrate (10 μC/mg) was dialyzed, and 10 ml reacted with one gm of sepharose 4B, activated with cyanogen bromide (Pharmacia). Other protein preparations, reacted with Affigel 10 (Biorad), were also found to be useful protease substrates. The bead-bound substrate was washed in 500 vol. of 0.01 M NaCl, 0.01 M Tris buffer, pH 7.2, and 0.0015 M $MgCl_2$ (RSB) at 4° to remove unreacted protein. The same coupling and washing procedure was used for preparation of protein substrates in all experiments. To the substrate (1.5 x 10^4 cpm in 0.1 ml) was added a known concentration of trypsin or HeLa cell extract (prepared by dounce homogenization in RSB of a HeLa cell suspension culture), in a final volume of one ml. The beads and enzyme were incubated with gentle agitation at 37° for 1-15 min, then 10 μl of 10% dodecyl sulfate were added to all samples, and the beads sedimented. Radioactive protein fragments released were determined by liquid scintillation counting of a portion of the supernate. A substrate control (no added enzyme) was always included to monitor the release of unreacted substrate, and any counts thus detected were subtracted from those samples with enzyme added.

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of a serine-type endoprotease in HeLa cells which could cleave poliovirus precursor polypeptides and other proteins (9,10). Assignment of a ribosomal site to such a protease is the purpose of this report.

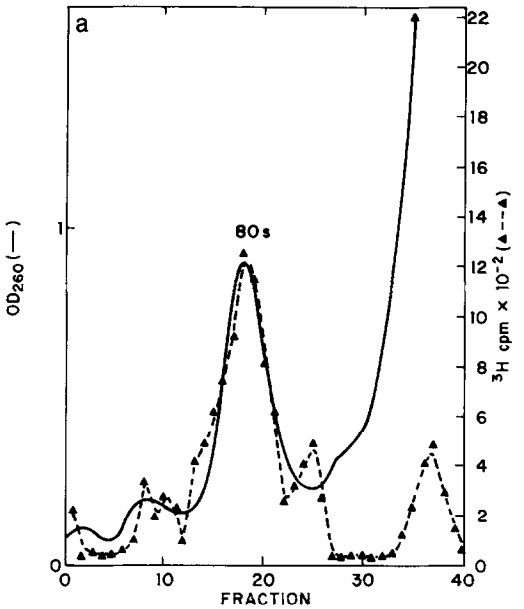

Figure 2a. Detection of protease activity in HeLa cell cytoplasm following zonal sedimentation. An extract of 1 x 10^8 HeLa cells was prepared, the nuclei removed, and the cytoplasm treated with 0.5% deoxycholate and 1% Triton X-100. The sample was layered over a 5-20% sucrose gradient containing RSB, and centrifuged for 120 min at 20,000 rpm at 4° in a SS-90 rotor in a Du Pont-Sorvall RC-2B centrifuge (sed. right to left). Fractions of 0.7 ml were collected, and protease activity was assayed by addition of 0.1 ml of ^{3}H-labeled HeLa cell proteins bound to Affi-gel 10 beads. The samples were incubated for 120 min at 35°, and released radioactivity determined.

Detection and quantitation of protease activity was facilitated by coupling radioactive substrates to an insoluble support. Proteolysis results in release of radioactive product from the support, and permits detection of as little as one picomole of bovine trypsin, and quantitation of neutral protease activity in extracts of 5 x 10^7 HeLa cells (Figure 1). The neutral protease activity in HeLa cells is able to cleave a variety of

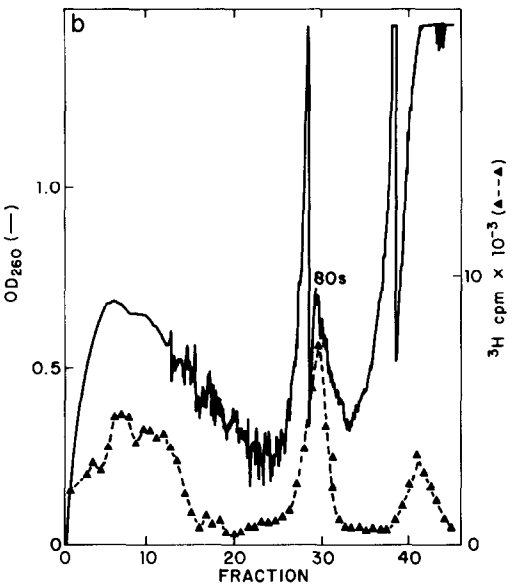

Figure 2b. As above, except that 2 x 10^8 cells were homogenized and the detergent-treated extract was centrifuged in a 5-47% sucrose gradient (56% sucrose cushion) containing RSB for 120 min at 4° in a SW-27 rotor in a Beckman L2-65 ultracentrifuge. Fractions of 0.8 ml were collected and measured for protease activity as in Figure 2a. Polysomes are present in fr. 2-20. Note that the absorbance scale changes over the monosome region; there are approx. 1.5 OD units in the peak fraction.

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protein substrates, including ^{125}I-labeled hemoglobin, gelatin, a mixture of ^{3}H-labeled HeLa cell proteins and viral precursor proteins. Increasing the substrate to extract ratio led, as expected for an enzymatic reaction, to release of more product. More than 70% of the products are TCA precipitable, indicating that the activity is endoproteolytic. The protease has a pH optimum of 8.0 on the substrates tested (10).

An interesting feature of the HeLa protease is that a part of the activity (up to 60%) sediments as a particulate in sucrose gradients, coinciding with cellular ribosomes (Figure 2a).

Ribosomes from extracts of cells prepared with detergents (0.5% deoxycholate and 1% Triton X-100), or without, possess similar levels of protease activity. Fifty micrograms of 80s ribosomal protein has protease activity equivalent to one μg of bovine trypsin. That implies that one of the seventy ribosomal proteins (avg. mol. wt. 30 Kd, ref. 11), has a specific activity of the order of trypsin. Ribosomes, purified by centrifugation to equilibrium in a discontinuous sucrose gradient retained their specific protease activity (not shown). Polyribosomes, prepared by zonal (Figure 2b) and isopycnic centrifugations, possess specific proteolytic activity at least that of the 80s ribosomes, but varying to higher levels. The results of zonal and equilibrium sedimentation with detergent treated extracts indicate the protease is attached to ribosomes, not membrane-bound structures.

In an attempt to correlate ribosome structure and function with the protease activity, several biological or biochemical treatments which affect ribosomes or protein synthesis were surveyed for their effects on ribosomal protease activity. The results are summarized in Table I. In general, treatments which damage or dissociate ribosomes, e.g. freeze-thaw or EDTA addition; or inhibit the process of translation, e.g. poliovirus infection or addition of double-stranded RNA, inhibit protease activity. The protease activity is less stable at 37° than at 25°, and is stimulated by addition of dithiothreitol. The action of the protease is inhibited by KCl, at levels which are often used in

TABLE I

Protease Activity of HeLa Cell 80s Ribosomes Following Various Treatments

Treatment [a]	Specific Protease Activity (% of Control Ribosome Preparation)
None	100
Dithiothreitol (5 mM)	188
Incubation at 25°	130
Freeze-thaw (2x, -20° to +37°)	7
EDTA (0.01 M)	5
Poly r·I-r·C (30 μg/ml)	40
Poly r·I-r·C (100 μg/ml)	7
KCL (50 mM)	50
KCl (100 mM)	20
Poliovirus infection[b]	20-40
TLCK (1×10^{-4} M)[c]	30
TPCK (1×10^{-4} M)[c]	83
Basic Pancreatic Trypsin Inhibitor (5×10^{-5} M)	2
Human α_1A Trypsin Inhibitor (2×10^{-5} M)	55

[a] All treatments were made on sucrose gradient-purified ribosomes, except where noted. Substrates were ^{125}I-labeled hemoglobin or ^{3}H-labeled HeLa cell proteins bound to sepharose 4B. Released radioactivity was determined to quantitate protease activity. Incubations of substrate and enzyme were for 120 min at 37°.

[b] HeLa cells were infected with 10^4 particles of poliovirus type 2 per cell. Incubation was at 35°. Ribosome preparations were made from mock-infected and infected cells after two hours of infection.

[c] TLCK, N-α-tosyl-L-lysylchloromethane; TPCK, tosyl-L-phenylalanyl chloromethane, both from Sigma Chemical Co.

cell-free protein synthesis reactions. This result may explain why protein cleavage during *in vitro* translation is not observed with certain messages (12). The specificity of the protease is

trypsin-like, judging from its inhibition by pancreatic trypsin inhibitor, α_1A trypsin inhibitor, and TLCK.

The question of whether the protease is loosely associated or an intrinsic ribosomal protein is being pursued. Assignment of a proteolytic role to a ribosome-associated protein would explain the rapid cleavages of nascent chains during translation of some messages in vivo (13) and in vitro (14), and the inhibition of translation initiation by protease inhibitors (15,16). Identification of the protease could serve as an additional useful ribosomal marker. It is worth noting that intracellular neutral proteases have been detected and assigned tentative regulatory roles in eucaryotic cells other than HeLa cells (6,8,17,18).

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