Intracellular protein degradation in serum-deprived human fibroblasts

Leif Andersen SLOT, Anne-Marie B. LAURIDSEN and Klavs B. HENDIL*

August Krogh Institute, 13 Universitetsparken, DK-2100 Copenhagen O, Denmark

IMR90 human fibroblasts were labelled by incubation of cells for 48 h in medium containing 10% serum and [^3H]leucine. The labelled protein was degraded at a rate of 1%/h during a subsequent incubation in medium with 10% serum. Incubation in medium without serum caused a transient enhancement of the degradation of endogenous protein, which was also found in cells labelled in medium without serum. The degradation of micro-injected haemoglobin was enhanced by serum deprivation in a non-transient manner. These results suggest that enhanced degradation in serum-free medium occurs only for a subpopulation of cell proteins and that it appears transient because the major part of the pool of susceptible endogenous proteins is being degraded during the first 20–30 h in serum-free unlabelled medium. Protein turnover in various cell compartments was measured by a double-labelling technique. Most of the enhanced degradation in serum-deprived cultures (73–83%) was due to breakdown of cytosolic proteins. The enhanced degradation of cytosolic proteins seemed to affect several proteins irrespective of their molecular mass or metabolic stability.

INTRODUCTION

Mammalian cells in culture are usually grown in a medium supplemented with serum. Deprivation of serum causes cell growth to halt and enhances the degradation of intracellular protein (Goldberg & St. John, 1976; Herskho & Ciechanover, 1982). Enhanced protein degradation is also found in liver cells starved of certain amino acids or if they are exposed to glucagon (Mortimore & Pösö, 1984).

The mechanisms of intracellular protein degradation are poorly understood. Most of the basal protein degradation in growing fibroblasts seems to take place in the cytosol (Bigelow *et al.*, 1981; Herskho & Ciechanover, 1982). However, enhancement of proteolysis is blocked by inhibitors of lysosomal function and is accompanied by morphological and biochemical changes in the lysosomes (Dean, 1980; Mortimore, 1982). The uptake into lysosomes of inert materials injected into the cytoplasm is also increased under conditions with enhanced protein degradation (Hendil, 1981; Seglen & Gordon, 1984). Enhanced degradation is therefore believed to consist of an uptake of cell components into the lysosomes (autophagy) and their subsequent degradation.

Basal proteolysis of intracellular proteins obviously is selective, as the half-times for degradation of specific proteins range from minutes to days (Goldberg & St. John, 1976). It has been debated whether the lysosomal uptake of proteins is also selective (Dean, 1984). Evidence is presented here that only a subset of proteins in human fibroblasts is affected by enhanced degradation. Most of these proteins are cytosolic. The selectivity of enhanced proteolysis is, however, shown to be limited within the soluble proteins: cytosolic proteins of different molecular mass and metabolic stability showed similar increases in degradation rate during serum deprivation.

EXPERIMENTAL

Materials

Plastic utensils for cell culture were obtained from Nunc (Roskilde, Denmark). The growth medium was Eagle's minimal essential medium with non-essential amino acids (Eagle, 1959) and 1 mM-pyruvate. It was prepared from the ingredient salts, amino acids (BDH, Poole, Dorset, U.K.) and vitamins (Merck, Darmstadt, Germany) or from powder medium (Gibco, Paisley, Scotland, U.K.). Newborn-calf serum and fetal-calf serum were also purchased from Gibco. L-[4,5- ^3H]Leucine, L-[U- ^{14}C]leucine and K ^{14}CNO were obtained from Amersham International (Amersham, Bucks, U.K.). Sendai virus was grown in embryonated eggs, purified by differential centrifugation and inactivated with β -propiolactone (Serva, Heidelberg, Germany) by standard methods (Giles & Ruddle, 1973). Phosphate-buffered saline as used here contains 137 mM-NaCl, 2.7 mM-KCl, 4.1 mM- Na_2HPO_4 and 0.73 mM- KH_2PO_4 , pH 7.4. Tris/saline refers to 150 mM-NaCl, 20 mM-Tris, 2 mM- MnCl_2 , 2 mM-glucose, pH 7.4. Biochemicals were purchased from Sigma (St. Louis, MO, U.S.A.) unless otherwise stated.

Cell culture

IMR90 human embryonic fibroblasts, initially purchased from Flow Laboratories (Irvine, Scotland, U.K.), were grown at 37 °C in medium with 10% serum [8% (v/v) newborn-calf serum plus 2% (v/v) fetal-calf serum]. Cells were used for experiments at population doubling levels between 30 and 35.

Degradation of endogenous proteins

All experiments were performed at 37 °C. Confluent cultures in 25 cm 2 flasks were labelled for 48 h in 4 ml of growth medium with 0.4 mM-[^3H]Leucine (5 $\mu\text{Ci/ml}$).

* To whom correspondence should be addressed.

The cells were then washed four times in phosphate-buffered saline with 0.4 mM unlabelled leucine and 0.1% serum. Fresh medium (9 ml) without or with 10% serum and with 4 mM unlabelled leucine was added to each culture to avoid re-incorporation of labelled leucine. Samples (1 ml) of medium were withdrawn from each flask at intervals thereafter. All medium was aspirated after the last sampling and the cell layer was dissolved in 2 or 4 ml of 0.1 M-NaOH/0.4% (v/v) sodium deoxycholate. Total radioactivity in 400 μ l samples of medium or cell lysate was counted in 2.5 ml of Picofluor 30 (Packard, Downers Grove, IL, U.S.A.). For determination of trichloroacetic acid-soluble radioactivity, samples (400 μ l) of medium or cell lysate were mixed with 100 μ l of bovine serum albumin (50 mg/ml) and 250 μ l of 25% (v/v) trichloroacetic acid. After centrifugation for 3 min at 12000 g, 400 μ l samples of the supernatants were counted for radioactivity in 2.5 ml of Picofluor 30. Counting efficiencies were determined by internal standardization.

Control experiments showed the same radioactivity (d.p.m.) for samples of cell lysate in NaOH as for samples solubilized by neutralization and digestion with Pronase (Merck) before radioactivity counting (Silverman *et al.*, 1985).

The degradation of intracellular protein was estimated as the increase in trichloroacetic acid-soluble radioactivity in the medium with time. It was calculated as percentage of initially incorporated radioactivity, which is the total recovered radioactivity minus the trichloroacetic acid-soluble radioactivity at the first sampling.

Labelling and micro-injection of haemoglobin

Human venous blood was drawn into heparin, oxygenated by shaking and centrifuged (1300 g, 10 min). The plasma and buffy coat were aspirated and the pellet was washed by centrifugations in 4 $\times$ 10 ml of 110 mM-NaCl/40 mM-sodium phosphate buffer, pH 6.5. A 100 μ l sample of the final pellet of red blood cells was mixed with an equal volume of 19 mM-K¹⁴CNO (52 mCi/mmol) in the same buffer and incubated at 37 °C for 5 h. The erythrocytes were then washed four times as described above in 5 mM-KCl/110 mM-NaCl/40 mM-sodium phosphate buffer, pH 8.0, and stored overnight at 4 °C. After this procedure, label is almost exclusively found in N-carbamoyl-haemoglobin (Hendil, 1980). IMR90 fibroblasts were removed with trypsin from confluent cultures, and 10⁷ were mixed with the labelled erythrocytes. The cells were washed in 4 $\times$ 10 ml of Tris/saline, and fused (Schlegel & Rechsteiner, 1978) by added 400 haemagglutinating units of β -propiolactone-inactivated Sendai virus. The cells were distributed into six 25 cm² Petri dishes and incubated at 37 °C for 4 h before they were washed in phosphate-buffered saline to remove all non-fused erythrocytes. Degradation of haemoglobin was determined as described for endogenous proteins.

Double isotope labelling

Dense cultures of IMR90 fibroblasts in 175 cm² flasks were labelled for 48 h in 30 ml of growth medium with 10% serum and 0.05 mM-[³H]leucine (1 μ Ci/ml). The cultures were washed in phosphate-buffered saline and incubated for additional 20–22 h in 50 ml of growth medium with 4 mM unlabelled leucine with or without 10% serum. Other cultures were labelled for 48 h in medium with 10% serum and 0.05 mM-[¹⁴C]leucine

(0.2 μ Ci/ml) but without subsequent incubation in unlabelled medium. The ¹⁴C- and ³H-labelled cells were then released from the culture flasks by 0.5 mM-EDTA in phosphate-buffered saline, mixed, and washed by centrifugations (1300 g, 5 min) in 2 $\times$ 10 ml of ice-cold phosphate-buffered saline before homogenization.

Cell fractionation: differential centrifugation

All procedures were carried out at 0–4 °C. The cells (about 50 mg) were homogenized in 4 ml of 250 mM-sucrose/5 mM-EDTA, pH 7.5, by 20 strokes in a tight-fitting Dounce homogenizer. In one experiment the sucrose solution also contained 0.5 mM-phenylmethanesulphonyl fluoride, 1 mM-iodoacetic acid, 0.1 mM-pepstatin and 2% (v/v) propanol. The homogenate was centrifuged at 290 g ($r_{av.}$ = 26 cm) for 10 min in a MSE Mistral 6L centrifuge to sediment the N fraction. As most nuclei are broken during homogenization, the N fraction consists mostly of intact cells and debris. All subsequent centrifugations were carried out in a MSE 10 $\times$ 10 rotor ($r_{av.}$ = 6.5 cm). Pellets were resuspended in 1.5 ml of sucrose for analysis. The postnuclear supernatant was centrifuged at 29000 g for 5 min to sediment the ML fraction. The fluffy layer on top of the pellet was aspirated together with the supernatant and re-centrifuged at 116000 g for 35 min to sediment the P fraction. The supernatant was centrifuged for 90 min at 167000 g to give the R fraction and the final supernatant, S.

Gradient centrifugation

The postnuclear supernatant, obtained as described above, was centrifuged in the MSE 10 $\times$ 10 rotor at 116000 g for 35 min. The pellet, equivalent to the MLP fraction, was resuspended in 1.5 ml of 250 mM-sucrose/5 mM-EDTA, pH 7.5. For density centrifugation, 10 ml tubes were filled successively with 0.5 ml of 67% (w/v) sucrose, 7 ml of gradient medium and 0.5 μ l of MLP fraction. The gradient medium contained 250 mM-sucrose, 0.7 mM-imidazole buffer, pH 7.0, and 30% (v/v) Percoll (Pharmacia, Uppsala, Sweden). The tubes were centrifuged at 30300 g ($r_{av.}$ = 5.6 cm) for 35 min in the 10 $\times$ 10 rotor before the contents were divided into fractions and weighed. A 200 μ l constriction pipette was used as a pycnometer for determination of densities.

Gel electrophoresis

Protein was precipitated from supernatant cell fractions with 10% trichloroacetic acid and washed with 3 $\times$ 1 ml of ethanol/diethyl ether (1:1, v/v). Precipitates were air dried, dissolved in SDS sample buffer with mercaptoethanol, and boiled. Electrophoresis was performed in 10% (w/v) - polyacrylamide tube gels (Laemmli, 1970). The gels were either stained with Coomassie Blue and scanned at 750 nm or frozen and sliced. Slices were swollen in 0.7 ml of Soluene 350 (Packard) in glass vials and counted for radioactivity in 10 ml of 0.5% (w/v) diphenyloxazole in toluene. Counting efficiencies for ³H and ¹⁴C were determined by internal standardization.

Other methods

Protein was determined as described by Lowry *et al.* (1951), with bovine serum albumin as standard. RNA was determined fluorimetrically (Morgan *et al.*, 1979). Marker enzymes for cell fractions and assay methods were as follows: cytochrome oxidase (Cooperstein & Lazarow, 1951), NADH-cytochrome c reductase (Hoge-

boom & Schneider, 1950), lactate dehydrogenase (Wroblewski & LaDue, 1955) and acid phosphatase (Robinson & Wilcox, 1969).

RESULTS

IMR90 cells were labelled with [^3H]leucine for 2 days in growth medium with 10% serum. The subsequent degradation of labelled protein showed first-order kinetics for at least 2 days when cells were kept in medium with 10% serum (Fig. 1). Deprivation of serum during the incubation in unlabelled medium enhanced protein degradation for 20–30 h, after which the rate of degradation decreased to a value comparable with that found in the serum-supplied cultures (Fig. 1). The enhancement may appear transient because only a subpopulation of proteins is affected and after 20–30 h most of this subpopulation has been degraded. Alternatively a degradative apparatus in the cells might be transiently activated. To explore these possibilities, some cultures were labelled for 24 h in medium containing serum, followed by 24 h in medium with the same specific radioactivity of [^3H]leucine but without serum. Again the subsequent degradation of protein showed first-order kinetics in medium with serum, and a transient enhancement of degradation was seen in cultures incubated without serum (Fig. 1). The cells remained viable, and less than 5% of the radioactive proteins were

released in trichloroacetic acid-soluble form during the experiments. If the initially high degradation rate in serum-free cultures was due to a transient activation of a proteolytic apparatus, the activation would have vanished during the labelling period in the serum-free medium, and no transiently enhanced degradation should have been seen during the subsequent incubation in unlabelled medium. The results therefore support the first explanation, assuming that serum deprivation stimulates the degradation of a limited pool of cell proteins.

This theory predicts that the degradation of any protein with a decreased stability as a result of serum deprivation would be persistently enhanced in the serum-free medium. To see if this was borne out, human haemoglobin was labelled and introduced into IMR90 fibroblasts by virus-mediated fusion between erythrocytes and fibroblasts. Fibroblast-to-fibroblast fusions were rare, as less than 2% of the nuclei were in bi- or multi-nucleated cells. As a result of the fusion, 0.2% of the radioactivity from the erythrocytes was injected into the fibroblasts. The medium was changed 18 h after the fusion to fresh medium with or without serum, and the degradation of intracellular haemoglobin was measured during the following 49 h. Fig. 2 shows that serum deprivation enhanced the degradation of haemoglobin for at least 49 h without transients. Similar results were obtained in three independent experiments. Less than 7% of the radioactivity in the microinjected cells was released into the medium in a trichloroacetic acid-insoluble form during the experiment. Doherty & Mayer (1985) have shown that cytosolic proteins erythrocyte-microinjected into mouse fibroblasts are largely sedimentable from cell homogenates. However, at least 90% of the haemoglobin injected was recovered in the supernatant fraction from

Fig. 1. Degradation of intracellular protein in IMR90 fibroblasts

Cells were labelled for 48 h in medium with [^3H]leucine and 10% serum (●, ○). The subsequent degradation of labelled protein was measured in medium without (○) or with (●) 10% serum. Other cultures (□, ■) were labelled for 24 h in medium with 10% serum and [^3H]leucine and then for further 24 h in medium without serum, before the degradation of protein was measured in unlabelled growth medium without (□) or with (■) 10% serum. The results are from one representative experiment. Each point represents the mean from three cultures. Error bars of 1 s.d. are shown when larger than the symbol. The curves were fitted to exponential functions as described in the text. The ordinate is on a logarithmic scale.

Fig. 2. Degradation of haemoglobin in IMR90 human fibroblasts

Haemoglobin was labelled by carbamoylation with K^{14}CNO and erythrocyte-microinjected into IMR90 cells. Degradation of haemoglobin was measured in growth medium with 10% serum (●) or without serum (○). The results are from one representative experiment out of three. The ordinate is on a logarithmic scale.

homogenates prepared 24 h after injection. Other experiments have shown (Hendil, 1980) that injection of haemoglobin has no deleterious effects on the recipient cells, that the degradation products are not re-incorporated, and that the degradation of haemoglobin is regulated in parallel with that of endogenous proteins. The experiments with haemoglobin reported here also support the theory that serum deprivation activates a proteolytic mechanism affecting a limited pool of intracellular proteins.

An estimate of the size of this protein pool was attempted by analysis of the data from the proteolysis of endogenous proteins (Fig. 1). The data were fitted to exponential functions by an iterative non-linear unweighed least-squares analysis (Wilkinson, 1961). Cell proteins are degraded according to first-order kinetics but differ in metabolic stability (Dean, 1980; Goldberg & St. John, 1976). However, the decay of cell bulk protein in cultures incubated in medium containing serum is approximately described by the exponential function $Y = 100e^{-mt}$, where Y is the amount (%) of protein remaining in the culture at time t and m is the decay constant (h^{-1}). Thus the continuous upper curve in Fig. 1 is described by $Y = 100e^{-0.011t}$. Enhancement of the degradation of a subpopulation of proteins is expected to result in a multiexponential decay. Provided that the decay constants for all proteins in the susceptible subpopulation are increased to the same extent, a biexponential function is obtained. As an approximation, the protein decay data from the serum-deprived cultures were therefore fitted to the equation

$$Y = Ae^{-kt} + (100-A)e^{-mt}$$

where A is a subpopulation of proteins (in %) whose decay is enhanced during serum-deprivation. k and m are the first-order decay constants for the two protein pools. Thus the lower continuous curve in Fig. 1 is described by $Y = 25e^{-0.056t} + 75e^{-0.010t}$. The size of the pool of proteins whose degradation becomes enhanced during serum deprivation (A) varied between $20 \pm 2\%$ and $35 \pm 6\%$ of the total in five independent experiments, with two to four cultures per group.

The lysosomes seem to be responsible for the enhanced degradation, since 0.1 mM-chloroquine, a lysosomotropic amine, abolished the enhanced degradation in serum-free medium (results not shown). Similar results have been reported previously (Dean, 1980).

We have attempted to identify the subpopulation of proteins whose degradation is enhanced by serum. Protein stabilities were assessed by a double-labelling technique (Arias *et al.*, 1969): cultures were labelled for 2 days in medium with [^3H]leucine and 10% serum before they were incubated for 20–22 h in unlabelled medium with or without serum to allow degradation of a fraction of the ^3H -labelled proteins. Some of the cultures were used to assess the extent of protein degradation as in the experiments described above. Other cultures were released from the culture flasks with EDTA and mixed with similar cultures labelled with [^{14}C]leucine but without a subsequent incubation in unlabelled medium. The cells were homogenized and fractionated by differential centrifugation. The ^{14}C labelling of a protein is taken as an indication of the relative amount of the protein present at the end of the labelling period. The isotope ratio in each fraction was corrected to equal initial radioactivities of ^3H and ^{14}C in the cells, as

calculated from the degradation in parallel cultures. The normalized $^3\text{H}/^{14}\text{C}$ ratio in any protein is then equal to the fraction of that particular protein remaining undegraded after incubation of the ^3H -labelled cells in unlabelled medium (Glass & Doyle, 1972). Fig. 3 shows the radioisotope ratios and distribution of marker enzymes, protein and RNA in the cell fractions. The R fraction is a high-speed pellet enriched in rRNA. The other fractions follow the classical designations (de Duve *et al.*, 1955), N, ML, P and S representing fractions enriched in nuclei, mitochondria and lysosomes, microsomal fraction, and supernatant, respectively. Cytochrome oxidase, acid phosphatase and lactate dehydrogenase are marker enzymes for mitochondria, lysosomes and cytosol respectively. NADH-cytochrome *c* reductase is found in both mitochondria and microsomal fractions (Remacle *et al.*, 1980). In Fig. 3(f) the normalized isotope ratios are plotted versus the cumulative contents of ^{14}C : the height of each block therefore indicates the proportion of protein remaining in the fraction, and the area of each block indicates the amount of protein remaining in the fraction after the chase period. The difference in protein degraded in cells in media with and without serum, i.e. the enhanced degradation, is illustrated by the hatched area in Fig. 3(f). In five independent experiments, 73–83% of the enhanced degradation was accounted for by proteolysis in the R and S fractions. Enhanced degradation of ribosomal protein and RNA in serum-deprived cells has been described by Sameshima *et al.* (1981). The preparations of soluble proteins are probably contaminated by nuclear proteins, since many nuclei rupture during homogenization. In a control experiment where the ^3H -labelled cells were harvested directly after the labelling period, the normalized $^3\text{H}/^{14}\text{C}$ ratios in the cell fractions varied between 0.98 and 1.02. The inclusion of phenylmethane-sulphonyl fluoride, iodoacetic acid and pepstatin in the homogenization buffer did not change the results. These inhibitors act on serine, cysteine and aspartate proteinases respectively (Barrett, 1980). Degradation of labelled proteins after homogenization is therefore not a significant source of error.

Combined ML and P fractions were further analysed by centrifugation in self-forming Percoll density gradients. Fig. 4 shows that the metabolic stability of proteins in the mitochondria-rich fractions were hardly affected by serum deprivation. However, lysosome-rich fractions (0–2 ml, Fig. 4) showed an apparent increase in degradation rate in serum-free cultures. This may be an artefact, since ^3H -labelled lysosomes from serum-deprived cells may be more fragile during homogenization than the smaller lysosomes from serum-supplied ^{14}C -labelled cells (Mortimore, 1982). This might lead to a loss of ^3H -labelled proteins from the lysosomes that is not due to protein degradation. Cell proteins in the low-density fractions (4.5–7.5 ml, Fig. 4) are less stable in serum-deprived than in serum-supplied cultures. Contamination of these fractions with soluble proteins can hardly account for the result, since the MLP fraction analysed in Fig. 4 contained less than 3% of the soluble lactate dehydrogenase in the homogenate, whereas about 23% of the total protein in the MLP pellet was recovered in the fractions at 4.5–7.5 ml in Fig. 4. These fractions were not further analysed.

Supernatant proteins from cell fractionations like those in Fig. 3 were separated by polyacrylamide-gel electro-

Fig. 3. Distribution of enzymes, RNA, and protein stability in subcellular fractions obtained by differential centrifugation

IMR90 cells were labelled for 48 h in medium with [^3H]leucine and 10% serum, before they were washed and incubated for 23 h in unlabelled medium without or with 10% serum. The cells were then mixed with other cells that had been similarly labelled but with [^{14}C]leucine and without a subsequent incubation in unlabelled medium. The cells were homogenized and divided into five fractions: N, ML, P, R and S (left to right). The enzyme distributions (a–e) are shown by diagrams (de Duve *et al.*, 1955) where the width of each block is proportional to the ^{14}C content in the fraction (distributions of ^{14}C and protein were almost identical). The ordinate in (a)–(e) shows the relative specific activity [% of activity or RNA divided by % of ^{14}C (protein)]. Recoveries of enzymes and RNA was between 85 and 117%. (a) Cytochrome oxidase; (b) RNA; (c) NADH–cytochrome *c* reductase; (d) acid phosphatase; (e) lactate dehydrogenase. In (f) the ordinate shows the $^3\text{H}/^{14}\text{C}$ ratio corrected to equal initial incorporation, i.e. the relative amount of ^3H -labelled protein that remains undegraded after incubation of cells in medium with (—) or without (---) serum. The hatched area represents the difference in protein degradation between cells incubated with and without serum.

phoresis. The gels were sliced and counted for radioactivity. The $^3\text{H}/^{14}\text{C}$ ratio in each slice was normalized to equal initial radioactivity, as described above, so that the normalized isotope ratio in each gel was identical with

that for the supernatant preparation analysed in the gel. The degradation constant, k_d , for the protein in each slice was calculated from $k_d = \ln(^3\text{H}/^{14}\text{C})/t$, where $^3\text{H}/^{14}\text{C}$ is the normalized isotope ratio in the slice, and t is the time

Fig. 4. Distribution of enzymes, protein, and protein stability in subcellular fractions obtained by density-gradient centrifugation

Cells were labelled and homogenized as described for Fig. 3. Fractions that are equivalent to combined ML and P fractions in Fig. 3 were separated in Percoll gradients. (a) ■, Acid phosphatase; ▲, cytochrome oxidase; △, NADH-cytochrome *c* reductase; ----, density. (b) $^3\text{H}/^{14}\text{C}$ ratios corrected to equal initial radioactivities. ^3H -labelled cells were incubated in medium with 10% serum (●) or without serum (○). □, ^{14}C radioactivity (protein).

for which the ^3H -labelled cells were incubated in unlabelled medium before harvest. Fig. 5(a) shows an optical scan of a stained gel and Fig. 5(b) shows the decay constants for proteins in the gels. Each gel slice contains several proteins. Nevertheless, the degradation of cytosolic proteins from cells kept in medium with serum varied between gel slices from about 0.20%/h to about 1.9%/h, with no apparent systematic variation with protein molecular mass. The curves in Fig. 5(b), which describe the variations in degradation constants in serum-deprived and serum-supplied cultures respectively, run almost parallel. The deviation from perfect parallelism may be due to the limited resolution in electrophoresis and gel slicing as well as imperfect alignment of the two gels. The experiment therefore gives the impression that serum deprivation increases the degradation of cytosolic proteins to similar extents, independent of molecular mass or the metabolic stability of the protein in question. Similar results were obtained in three independent experiments, one of them with proteinase inhibitors (phenylmethanesulphonyl fluoride, iodoacetic acid and pepstatin) present in the homogenization buffer.

DISCUSSION

Deprivation of serum increases the degradation of both endogenous (Amenta *et al.*, 1976; Dean, 1980) and microinjected (Hendil, 1980; Neff *et al.*, 1981; Backer *et al.*, 1983; Rote & Rechsteiner, 1983) proteins in several cell lines. The enhanced degradation of endogenous proteins is seen for only about 30 h (Fig. 1). Similar results have been reported by others (Amenta *et al.*, 1976; Castor, 1977; Cockle & Dean, 1982; Auteri *et al.*, 1983). Our results suggest that the enhanced degradation seems transient because it affects only a subset of proteins: once these are degraded, overall proteolysis becomes similar to the degradation in cells maintained in medium with serum. This is inferred from the lasting stimulation of degradation of microinjected haemoglobin (Fig. 2) and from the presence of a transient enhanced degradation in cells that had been labelled in serum-free medium (Fig. 1). The latter experiment also shows that the protein pool whose degradation is enhanced by serum deprivation is continuously synthesized in serum-free medium, so that its turnover rate has increased.

Fig. 5. Electrophoretic pattern of degradation rates of cytosolic proteins

Proteins from the supernatant fractions shown in Fig. 3 were separated by SDS/polyacrylamide-gel electrophoresis. (a) Densitometric profile of Coomassie-Blue-stained gel. Arrows indicate positions of protein M_r standards (100, phosphorylase *b*; 68, bovine serum albumin; 43, ovalbumin; 29, carbonic anhydrase; 18.4, β -lactoglobulin). (b) First-order degradation constants for cytosolic proteins from cells incubated in medium with 10% serum ($\bullet$) or without serum ($\circ$). The degradation constants were calculated from the $^3\text{H}/^{14}\text{C}$ ratio in each gel slice, as described in the text. The S.D. is expected from counting statistics to be less than $\pm 0.0005 \text{ h}^{-1}$.

In human fibroblasts, most of the proteins whose metabolic stability is decreased by serum deprivation are found in the supernatant fraction (Fig. 3). This may not be universal. Enhanced degradation is thought to involve the uptake of cell components into lysosomes, and in conditions with enhanced degradation hepatic lysosomes contain mitochondria and other cell elements in roughly the same proportions as in the cytoplasm (Mortimore & Pösö, 1984).

Like Russell *et al.* (1981), we found no systematic correlation between the metabolic stability of soluble proteins and their molecular mass (Fig. 5). Others have found, perhaps because of different experimental conditions, that small proteins tend to be more stable than large ones (Dice *et al.*, 1973; Goldberg & St. John, 1976).

The degradation of proteins follows first-order kinetics (Dean, 1980; Goldberg & St. John, 1976). An unselective uptake of cytoplasmic proteins into lysosomes in enhanced degradation is expected to increase the first-order degradation constants for any cytoplasmic protein from k_d to $(k_d + k_1)$, where k_1 is the fractional hourly uptake of cytoplasm into lysosomes. In keeping with this theory, our gel electrophoresis experiments suggest that the enhancement of degradation varies little among proteins in the gel slices (Fig. 5b). The concept of mainly unspecific lysosomal uptake of cytoplasm during enhanced degradation is also supported by the observations that sucrose (Seglen & Gordon, 1984) and dextran (Hendil, 1980) introduced into the cytoplasm become sequestered in lysosomes under conditions with enhanced protein degradation.

It has been argued that enhanced degradation is selective, since the degradation of long-lived proteins is relatively more affected than the degradation of short-lived proteins (Knowles & Ballard, 1976; Neff *et al.*, 1979). However, a low absolute increase in degradation rate is readily detectable on a background of low proteolysis but difficult to discern on a background of an already high degradation rate.

On the other hand, the degradation of small, basic, non-glycosylated proteins is preferentially enhanced in liver in diabetes (Samaniego *et al.*, 1981). Backer *et al.* (1983) have suggested that the enhanced degradation of soluble proteins is selective also in IMR90 fibroblasts because a certain protein region is necessary for the enhancement of degradation of microinjected ribonuclease A.

A non-selective enhancement of the degradation of cytosolic proteins would suggest that the pool of proteins that become less stable in serum-deficient medium amounts to about 50% of the total cell protein: this is the proportion of supernatant (S + R) proteins to total cell proteins (Fig. 3). However, the biexponential curve-fit for the degradation of bulk protein suggested that this pool constitutes only 20–35% of total cell protein (Fig. 1a). The discrepancy between these pool size estimates may be resolved if the degradation of all cytoplasmic proteins is actually enhanced to the same extent, but only in a subpopulation of cells. The idea that fibroblast cultures contain some cells that do not change their proteolysis on serum deprivation has been put forward by Amenta & Brocher (1980). However, the monoexponential degradation of haemoglobin in serum-deprived cells (Fig. 2) suggests that the cell population is homogeneous with respect to enhancement of protein degradation. Therefore perhaps only a subpopulation of cytosolic proteins in each cell is degraded more rapidly in serum-deprived cells. To resolve between these possibilities, it will be necessary to monitor the degradation of several individual cytosolic proteins.

In summary, our results suggest that the enhanced degradation in serum-deprived human fibroblasts involves lysosomes and that it affects only a subpopulation of cellular proteins. Most of these proteins are cytosolic, and they differ widely in M_r and metabolic stability.

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