Biochem. J. (1989) 258, 33–40 (Printed in Great Britain)

Vanadate inhibits degradation of short-lived, but not of long-lived, proteins in L-132 human cells

José Luis VARGAS, Fernando ANIENTO, Javier CERVERA and Erwin KNECHT*
Instituto de Investigaciones Citológicas de la Caja de Ahorros de Valencia,
Centro asociado del Consejo Superior de Investigaciones Científicas, Amadeo de Saboya 4, 46010-Valencia, Spain

Vanadate, at concentrations higher than 0.04 mM, inhibits the intracellular degradation of short-lived proteins in exponentially growing L-132 human cells. The inhibition is not due to a decrease in viability or in the ATP contents of the cells, Since vanadate decreases proteolysis in cell extracts, the inhibition appears to affect the proteinases which degrade these proteins. Under optimal nutritional conditions, the degradation of long-lived proteins is accelerated by vanadate, thus providing additional evidence that in exponentially growing cultured cells degradation of short- and long-lived proteins occurs by different processes. Vanadate also efficiently inhibits the lysosomal degradation of endocytosed proteins and of long-lived proteins under step-down conditions. However, this effect seems to be unrelated to the observed inhibition of degradation of short-lived proteins, because chloroquine and leupeptin, which inhibit degradation of proteins by lysosomes, do not modify the degradation of these proteins. Our results provide for the first time a probe which, owing to its opposite effects on the degradation of short- and long-lived proteins, could be useful to clarify the mechanisms involved in protein degradation in cultured cells.

INTRODUCTION

In eukaryotic and prokaryotic cells, measurements of the degradation of isotopically labelled proteins support the existence of two main groups, short-lived and long-lived proteins [1–4]. Although less than 5% of the cell proteins are short-lived, owing to their rapid turnover the energy needed for their synthesis represents more than 30% of the total consumed by the cell [5–7]. However, neither the identity of the short-lived cellular proteins nor the mechanism(s) of their degradation have been fully established, probably because of the lack of specific agents which could modify the proteolytic rate of these proteins.

Such inhibitors have proved their usefulness in the study of the degradative mechanisms of proteins in hepatocytes and cultured cells under nutritional deprivation [3,8,9]. A systematic study was therefore made with chemicals which have been useful in inhibiting protein degradation in other biological systems [8], to find selective inhibitors of the degradation of short-lived proteins. We found that vanadate selectively inhibits degradation of short-lived proteins in exponentially growing cells, but accelerates the degradation of long-lived proteins.

EXPERIMENTAL

Chemicals

[1-^{14}C]Leucine (56 mCi/mmol; 0.05 Ci/l), [4,5-^{3}H]-leucine (52 Ci/mmol; 1 Ci/l) and [^{35}S]methionine (1000 Ci/mmol; 15 Ci/l) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Sodium orthovanadate, *p*-chloromercuribenzoic acid, EDTA, leupeptin, pepstatin, chymostatin, phenylmethanesulphonyl fluoride, *N*-α-*p*-tosyl-L-lysylchloromethane ('TLCK') hydrochloride, cytochalasin B, vinblastine, colchicine, cycloheximide, Tris and other biochemicals were from Sigma Chemical Co., St. Louis, MO, U.S.A.

Cells, media and salt solutions

L-132 cells (line derived from normal human embryonic lung; A.T.C.C. no. CCL 5; Flow Laboratories, Irvine, Scotland, U.K.) were grown in monolayer (25 cm^2 Falcon flasks; A/S Nunc, Roskilde, Denmark) in Minimum Essential Medium supplemented with Earle's salts, 10% (v/v) foetal-bovine serum ('growth' medium), 100 units of penicillin/ml and 100 μg of streptomycin/ml. In some experiments this medium was used without serum ('step-down' medium). Viability of cells was assessed by the Trypan Blue exclusion test, and cell growth was determined with a Coulter counter.

Measurements of protein synthesis

Experiments to measure synthesis of proteins were performed essentially as described previously [10]. Briefly, exponentially growing cells were incubated for 2 h at 37 °C in 'growth' medium containing 10 μCi of [^{3}H]-leucine/ml. The cells were then washed twice with ice-cold Dulbecco's phosphate-buffered saline without Ca^{2+} and Mg^{2+} (cat. no. 18-604; Flow Laboratories), containing 2 mM-leucine, twice with 10% trichloroacetic acid and dissolved, overnight, in 0.2 M-NaOH containing 0.4% sodium deoxycholate at 50 °C. Radioactivity incorporated into the protein present in the samples was measured by liquid-scintillation counting.

Measurements of protein degradation

Long-lived proteins were labelled by incubating cells for 24 h in 'growth' medium containing 1–2 μCi of [^{14}C]leucine/ml. The cells were then washed twice in

* To whom correspondence and reprint request should be addressed.

phosphate-buffered saline containing 2 mM-leucine. They were incubated for 16–20 h in 'growth' medium plus 2 mM-leucine, to chase short-lived proteins. To label short-lived proteins, cells were incubated for 15 min with 10 μCi of [^{3}H]leucine/ml, washed twice with phosphate-buffered saline containing 2 mM-leucine and incubated at 37 °C for 10 min, to chase free labelled amino acids from the intracellular pools [11], before starting protein-degradation measurements. For double-labelling experiments, exponentially growing cultured cells in 25 cm^2 flasks containing 5 ml of complete medium were incubated with 0.5 μCi of [^{14}C]leucine for 24 h to label long-lived proteins. This medium was replaced with fresh complete medium containing 2 mM-leucine to prevent incorporation into protein of labelled leucine released by proteolysis. After 16–20 h, the cells were washed twice with fresh complete medium and labelled in 5 ml of fresh complete medium containing 20 μCi of [^{3}H]leucine for 15 min to label short-lived proteins. ^{3}H and ^{14}C radioactivities were calculated separately, after correction for channel spill-over and quenching by the external-standard method (Beckman LS-7800 liquid-scintillation counter).

Measurements of intracellular protein degradation of short- and long-lived proteins were performed in medium with or without vanadate, as previously described [12]. Briefly, 0.5 ml portions of the medium were taken during the chase period, and 0.5 ml of 10% trichloroacetic acid was added to each portion. After 30 min in an ice bath, the samples were centrifuged for 10 min at 5000 *g*, and the pellet was washed with 0.5 ml of 5% trichloroacetic acid and centrifuged again. Supernatants from both centrifugations were pooled. The sediment was dissolved in 1 M-NaOH and neutralized with HCl. Radioactivity in supernatant and sediment was measured by liquid-scintillation counting. At the end of the chase period, the final radioactivity in the cells was determined as described for protein synthesis. In experiments with 'step-down' medium, bovine serum albumin (final concn. 3 mg/ml) was added as carrier to aid co-precipitation. The initial radioactivity was calculated by adding the final radioactivity in the cells to the total radioactivity released into the medium during the chase period. Trichloroacetic acid-precipitable radioactivity released into the medium was always less than 1% of the original radioactivity/h, and represents protein secretion and/or detachment of cells from the monolayer. Protein degradation of each chase time was calculated by dividing the total trichloroacetic acid-soluble radioactivity released at this time to the medium by the initial radioactivity in the cells (calculated as described above) and expressed as %. All results are means ± S.D. from three or more experiments.

Proteolysis in cell extracts

Short-lived proteins were labelled as described above. Flasks were washed three times with phosphate-buffered saline, 1 ml of ice-cold water was added and the cells were removed from the flasks by scraping. The cell suspensions were sonicated in an ice bath for 2 min (8 × 15 s pulses, with 30 s intervals between pulses) with a Braun Labsonic 1510 sonicator at 300 W. Cell extracts (0.2 mg of protein/ml) were incubated at 37 °C in 0.1 M-Mes, adjusted to pH 5.5 or 7.0. Portions (500 μl) were taken at 0, 1 or 2 h and processed as described for the measurements of protein degradation in living cells, with bovine serum albumin (final concn. 3 mg/ml) as carrier. Proteolysis was calculated at the different times from the ratio of the radioactivity in the trichloroacetic acid-soluble fraction (radioactivity in the acid-soluble fraction at zero time was considered to be nil) to the total radioactivity at this time and expressed as %.

Degradation of endocytosed proteins

Labelled proteins used for endocytosis were obtained by incubating L-132 cells for 24 h in 4 ml of 'growth' medium containing 0.1 mCi of [^{3}H]leucine/ml. The cells were washed three times with ice-cold phosphate-buffered saline containing 2 mM-leucine. Then they were scraped from the flasks, suspended in 1 ml of sample buffer (62.5 mM-Tris/HCl, 18 mM-2-mercaptoethanol, 35 mM-SDS and 1.7 M-glycerol, pH 6.8). The suspensions were sonicated as in the preparation of cell extracts, boiled for 5 min and centrifuged at 1000 *g* for 10 min. Supernatants were filtered through a PD-10 column (Pharmacia Fine Chemicals) equilibrated with phosphate-buffered saline. For endocytosis the cells were incubated for 60–75 min at 37 °C with 3 ml of the labelled proteins prepared as above. After washing, 6 ml of 'growth' medium was added to each flask, and degradation was calculated as described for endogenous labelled proteins.

General

Electron microscopy was carried out as described in ref. [13]. Briefly, cells were fixed in the flasks, or after detachment with trypsin, for 1 h at 4 °C with 2% glutaraldehyde in 0.1 M-phosphate buffer, pH 7.3, washed in this buffer and fixed for 1 h with 1% OsO_4 in 0.05 M-veronal acetate buffer, pH 7.3. The osmolarities of the last two solutions were adjusted, with sucrose, to 400 mosM. After dehydration in a graded series of acetones, cells were embedded in Vestopal W by standard procedures.

Proteins synthesized by L-132 cells, in the presence or absence of vanadate, were analysed by SDS/polyacrylamide-gel electrophoresis [14] and fluorography [15].

Biuret [16] and Lowry/deoxycholate [17] methods were used for protein measurements, with bovine serum albumin as standard.

ATP was measured by the firefly luciferin–luciferase reaction [18]. Cell monolayers were treated with 1 M-$HClO_4$, scraped from the flasks, sonicated (200 W, 1 min) and centrifuged for 10 min at 10000 *g*. Supernatants were neutralized and used for ATP measurements. The assay was linear from about 0.6 to 10 nM-ATP.

Lysosomes were obtained as described in ref. [19].

RESULTS

Effect of vanadate on the degradation of short- and long-lived proteins in exponentially growing cultured cells

In preliminary experiments a systematic study was made of the degradation of short-lived proteins with several chemicals which have shown their effectiveness in suppressing lysosomal and non-lysosomal degradation of proteins in hepatocytes [8] and other cells. These included agents that raise the intralysosomal pH (10 mM-NH_4Cl, 100 μM-chloroquine), proteinase inhibitors (100 μM-leupeptin, 100 μM-pepstatin, 100 μM-phenyl-

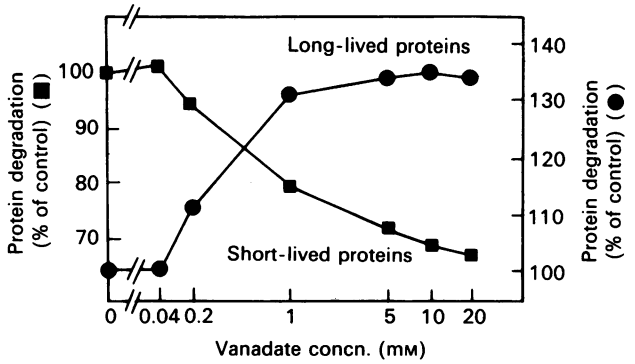

Fig. 1. Effect of different concentrations of vanadate on the degradation of short-lived (■) and long-lived (●) proteins

Proteins were labelled as described in the Experimental section and incubated without or with different amounts of vanadate. Protein degradation was measured at 1 and 2 h, and the values were averaged. Results, which are calculated from three or more different experiments, are shown as % of the degradation values without vanadate (%/h); S.D. values were always less than 10%. The absolute values of protein degradation for short- and long-lived proteins were 7.32 ± 0.51 and 0.59 ± 0.05% of initial radioactivity in the cells/h respectively (mean $\pm$ S.D. from six different experiments).

methanesulphonyl fluoride, 100 μM-chymostatin), cytoskeletal poisons (10 μM-vinblastine, 10 μM-colchicine) and protein-synthesis inhibitors (100 μM-cycloheximide). None of these agents decreased, in growing L-132 cells, the degradation rate of short-lived proteins below 90% of the value in control cells (results not shown), in good agreement with the results of others [3,9,10,20,21]. However, vanadate, which has been shown to affect protein degradation in hepatocytes [22], had a substantial effect on the rate of proteolysis. Fig. 1 shows that addition of vanadate (at concentrations higher than 0.04 mM) to L-132 cells affects, to the same extent but in opposite directions, the degradation of both short- and long-lived proteins. Vanadate inhibited degradation of short-lived proteins, with the maximum inhibitory effect (approx. 30%) at 10–20 mM-vanadate. With long-lived proteins, vanadate accelerated the degradation rate, with a nearly maximum effect at 1 mM. Similar results were obtained in double-labelling experiments (Fig. 2). Therefore, and hereafter unless otherwise indicated, all results refer to a 10 mM concentration of vanadate.

When cells treated with vanadate were preincubated in vanadate-free medium, not only was the inhibition of the degradation of short-lived proteins rapidly reversed, but an enhanced proteolysis of short-lived proteins was produced until all short-lived proteins were degraded (Fig. 3). This enhanced proteolysis was also observed with long-lived proteins (Fig. 4).

Viability, protein synthesis and morphology of vanadate-treated cells

Although at the concentrations used vanadate does not modify cell viability (98 ± 2% in control cells; 97 ± 2% in vanadate-treated cells), protein synthesis was impaired to some extent (incorporation for 2 h of [^{3}H]leucine in vanadate-treated cells was 28% that of control cells). However, the synthesized proteins appear

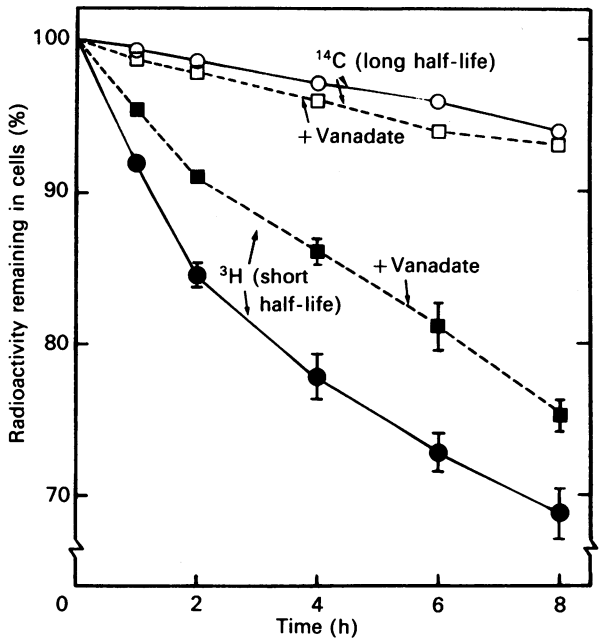

Fig. 2. Effect of 10 mM-vanadate on the degradation of short-lived and long-lived proteins labelled in the same monolayers of cells

Double labelling of cells with [^{3}H]leucine and [^{14}C]leucine and measurements of protein degradation were as described in the Experimental section. The results shown are means $\pm$ S.D. for four different flasks. Where S.D. values are not shown, they lie within the boundaries of the data points. Analysis by Student's t test indicated that vanadate inhibited degradation of short-lived proteins and accelerated degradation of long-lived proteins at every time point ($P < 0.05$). ^{3}H-labelled short-lived proteins: ●, control cells; ■, vanadate-treated cells; ^{14}C-labelled long-lived proteins: ○, control cells, □, vanadate-treated cells. Similar results were obtained when short-lived proteins were labelled with [^{14}C]leucine and long-lived proteins with [^{3}H]leucine.

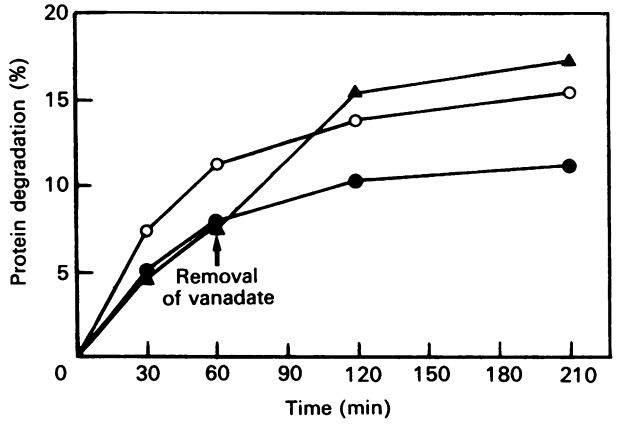

Fig. 3. Reversibility of the inhibitory effect of vanadate

Short-lived proteins were pulse-labelled and protein degradation was calculated as described in the Experimental section. Vanadate was used at a final concentration of 10 mM. ○, Control cells (no additions); ●; cells incubated with vanadate; ▲, cells incubated first with vanadate and, after 60 min (arrow), washed and further incubated with fresh medium without vanadate. Each point is the mean of four different experiments with duplicate samples. The S.D. values were 2–6% of the mean values.

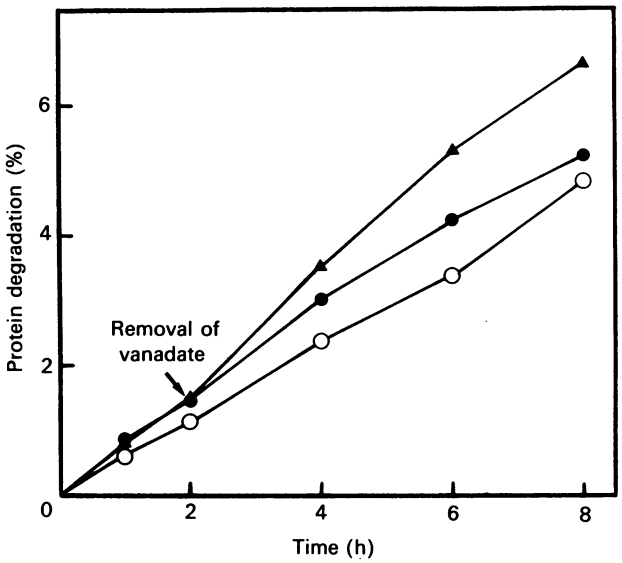

Fig. 4. Effect of removal of vanadate on the degradation of long-lived proteins

Long-lived proteins were labelled and protein degradation was calculated as described in the Experimental section. Vanadate was used at a final concentration of 10 mM. ○, Control cells (no additions); ●, cells incubated with vanadate; ▲, cells incubated first with vanadate and, after 60 min (arrow), washed and further incubated with fresh medium without vanadate. Each point is the mean of three different experiments with duplicate samples. The S.D. values were 3–8 % of the mean values.

to be the same, as judged by SDS/polyacrylamide-gel electrophoresis and fluorography (results not shown).

Vanadate also produces changes in the electron-microscopic appearance of L-132 cells: the vacuolar system (endoplasmic reticulum, Golgi apparatus and lysosomes) appears swollen (Fig. 5).

Intracellular ATP concentrations in vanadate-treated cells

Since vanadate is a phosphate analogue, there was the possibility that this chemical inhibits short-lived proteins by lowering intracellular ATP concentrations. However, under the conditions used here, a significant ($P < 0.01$) decrease in ATP by vanadate was only observed after 8 h of treatment (57±4% of control values).

Vanadate inhibition of intracellular proteinases in cell extracts

Vanadate inhibits lysosomal and some neutral proteinases [22–27], and therefore this chemical could be an inhibitor of the cell proteinases responsible for the degradation of short-lived proteins. As shown in Table 1, vanadate decreased the degradation of endogenous labelled proteins in cell homogenates. The degree of inhibition was 44% at acid pH (lysosomal proteinases) and 38% at neutral pH (neutral proteinases and lysosomal proteinases working at sub-optimal conditions). The effect of inhibitors of several groups of proteinases was also checked. At acid pH, the proteolysis in the cell homogenates was decreased by inhibitors of aspartic proteinases (pepstatin) and of cysteine proteinases (*p*-chloromercuribenzoate, leupeptin), whereas at neutral pH inhibitors of serine (phenylmethanesulphonyl fluoride, 'TLCK'), metallo- (EDTA) and cysteine proteinases were effective. The effect of vanadate on protein degradation was roughly additive to that produced by inhibitors of serine, metallo- and aspartic proteinases and not additive to that produced by cysteine-proteinase inhibitors. This suggests that this chemical modifies the activities of cysteine proteinases of L-132 cells.

It has been reported that vanadate inhibits some ATP-stimulated proteinases [23–27]. The effect of ATP on the proteolysis in cell homogenates was therefore tested. ATP increased the proteolysis at acid pH (lysosomal proteinases) and had a lesser effect at neutral pH; this increased proteolysis seems to be affected by vanadate. To demonstrate that the effect of vanadate at acid pH affects lysosomal proteinases, the proteolysis in the extracts after addition of broken lysosomes from rat liver was measured. As shown in Table 1, vanadate was also able to inhibit rat liver lysosomal proteinases.

Effects of vanadate on lysosomal degradation in cultured cells

Lysosomes are still the best-characterized locus of proteolytic activity in mammalian cells [3–5,8,9,28]. We therefore tested whether lysosomal degradation of

Table 1. Effects of vanadate and several proteinase inhibitors on the proteolysis of cell homogenates *in vitro*

Radioactively labelled homogenates were prepared as described in the Experimental section. Additions were made at the start of the 1 h incubation. Results are expressed as percentages of the values obtained in homogenates incubated in parallel with no additions. The absolute values of protein degradation were 2.1±0.2%/h at pH 5.5 and 1.1±0.1%/h at pH 7.0 (means±S.D. from three different experiments).

Additions	Degradation (%) pH 5.5	pH 7.0
None	100	100
Pepstatin (0.1 mM)	80	97
Leupeptin (0.3 mM)	52	58
p-Chloromercuribenzoate (0.3 mM)	48	64
Phenylmethanesulphonyl fluoride (0.1 mM)	92	55
'TLCK' (0.5 mM)	100	87
EDTA (1 mM)	98	90
ATP (3 mM)	124	115
Vanadate (1 mM)	56	62
Vanadate + pepstatin	39	61
Vanadate + leupeptin	42	59
Vanadate + *p*-chloromercuribenzoate	43	45
Vanadate + phenylmethanesulphonyl fluoride	55	31
Vanadate + 'TLCK'	56	48
Vanadate + EDTA	48	45
Vanadate + ATP	55	65
Broken lysosomes (0.024 unit of β-*N*-acetylglucosaminidase/ml)	1366	–
Vanadate + broken lysosomes	701	–

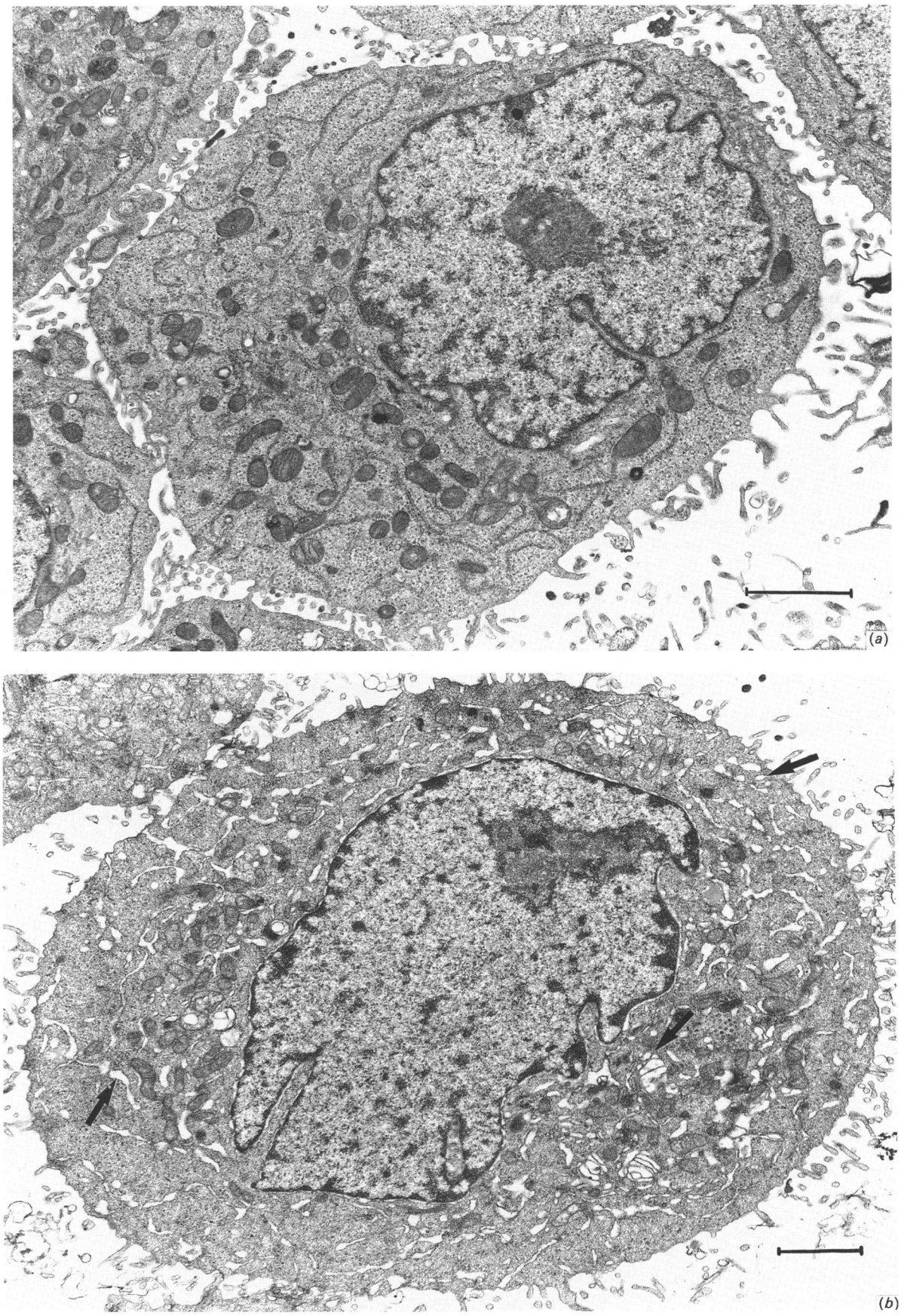

Fig. 5. Effect of vanadate on the electron-microscopic appearance of L-132 cells

L-132 cells were incubated in 'growth' medium for 2 h without (*a*) or with (*b*) 10 mM-vanadate. Note the swollen appearance of the vacuolar system (arrows) in vanadate-treated cells (*b*). Bar represents 2 μM.

Table 2. Effects of vanadate on the degradation of endocytosed proteins

Incorporation of labelled proteins into cultured cells via endocytosis and measurements of protein degradation were carried out as described in the Experimental section. Results are expressed as means ± S.D. for four experiments.

	Protein degradation (%)	
Time (h)	Control (no additions)	+10 mM-vanadate
0.5	14.9±1.5	3.1±0.2
1	24.5±2.0	5.3±0.4
2	34.0±2.1	8.7±0.7

Table 3. Effect of vanadate on the proteolytic rate of long-lived proteins of L-132 cells under various growth conditions

Proteolytic degradation was measured as described in the Experimental section at 1, 2, 4, 6 and 8 h, and proteolytic rates per h were calculated from these values by least-squares regression. Results are shown as means ± S.D. of three different experiments with duplicate samples.

	Proteolytic rate (% of trichloroacetic acid-soluble radioactivity released/h)	
Growth conditions	No additions	+vanadate
Exponential growth	0.57±0.02	0.70±0.04
Serum deprivation	1.05±0.07	0.66±0.04
Confluent culture	0.67±0.02	0.75±0.07
Confluent culture plus serum deprivation	1.13±0.02	0.72±0.03

proteins is affected by vanadate, taking advantage of the fact that lysosomes are implicated in the degradation of endocytosed proteins [28] and of intracellular long-lived proteins under nutritional deprivation ('step-down' conditions) [20,28,29]. Vanadate inhibited both the degradation of endocytosed proteins (Table 2) and the increased degradation observed under step-down conditions (Table 3). The inhibitory effect observed under step-down conditions is due to the absence of serum from the medium, and not to differences between growing and non-growing cells, since the same effects of vanadate on the degradation of long-lived proteins can be observed with confluent cultures (i.e. stimulation of degradation of long-lived proteins in the presence of serum and inhibition of degradation of long-lived proteins in the absence of serum; Table 3). After incubation for 8 h with vanadate, the degradation rate of long-lived proteins under growing conditions decreased (results not shown), possibly owing to the decrease in the ATP content (see above).

Table 4. Combined effects of vanadate, centrifugation and NaF on the rate of degradation of short-lived proteins

The isotopic labelling of cell proteins and the measurements of protein degradation were as indicated in the Experimental section. Vanadate and NaF (both at 10 mM final concn.) were added at the beginning of the chase. Centrifugation was carried out exactly as previously described [30]. The values are means ± S.D. for three experiments. Numbers in parentheses indicate the degradation expressed as percentage of that in untreated cells. The ATP contents of cells treated for 60 min with NaF were 34–43% of controls.

Treatment	Degradation of short-lived proteins (%/h)
None	7.7±0.6 (100)
Vanadate	5.2±0.5 (68)
Centrifugation	2.9±0.1 (38)
NaF	4.7±0.3 (61)
Vanadate + centrifugation	1.0±0.1 (13)
Vanadate + NaF	4.2±0.2 (54)
Centrifugation + NaF	1.9±0.1 (25)

Combined effect of vanadate, centrifugation and NaF on the degradation rate of short-lived proteins

It has been reported that graded decrements in the cellular ATP content (with inhibitors of energy metabolism) caused a graded decrease in the rates of proteolysis in fibroblasts [10] and that centrifugation of cells inhibits selectively the degradation of short-lived proteins [30]. Therefore, to clarify if vanadate affects the same degradative mechanisms, the combined effects of centrifugation, vanadate and NaF were studied. As shown in Table 4, the inhibitions by centrifugation and vanadate (but not by NaF and vanadate) were additive.

DISCUSSION

In exponentially growing cultured cells, proteins (long-lived, short-lived or micro-injected) seem to be degraded mostly by unknown non-lysosomal mechanisms, because agents which affect lysosomal functions produce little, if any, modifications in their proteolytic rate [31,32]. Although the degradation of both protein groups has similar ATP requirements [10] and is sensitive to lowered temperatures [7] and metabolic poisons [1,20], the degradative mechanisms for short- and long-lived proteins appear to be different because, in contrast with the degradation of long-lived proteins, degradation of short-lived proteins: (*a*) does not increase under nutritional deprivation [3,12,33–35]; (*b*) is less affected by low temperatures [10]; and (*c*) is selectively inhibited by centrifugation [30]. The present study shows that degradation of short-lived proteins, but not of long-lived proteins, in exponentially growing cells is specifically inhibited by vanadate, suggesting again that at least some of the steps in the degradation of these two classes of proteins in exponentially growing cells may be different.

The effects of vanadate on the degradation of long-lived proteins (Figs. 1 and 2), the reversibility of the

inhibition of the degradation of short-lived proteins (Fig. 3), as well as the effects of vanadate on cell viability, indicate that the observed inhibition of the degradation of short-lived proteins is not due to a toxic effect of vanadate. Also, the possibility that the observed inhibition of degradation could be due to the lack of (*a*) newly synthesized protein(s) necessary for this degradation appears unlikely, because addition of cycloheximide to the medium, in the presence or absence of vanadate, does not by itself change the degradation of short-lived proteins (results not shown). These results confirm and extend previous results obtained with several inhibitors of protein synthesis [9,36]. Since the inhibition of protein degradation was observed at early incubation times (30 min), we also conclude that the effect of vanadate on protein degradation during the early chase times was not mediated by a decrease in the ATP concentration, which only occurs after a long exposure to vanadate. This possibility is further unlikely, because it has been found that a decrease in ATP inhibits degradation of both short- and long-lived proteins to the same degree [10], whereas vanadate, during the chase period studied, inhibited only degradation of short-lived proteins (Figs. 1 and 2). It does not seem probable that vanadate inhibits the marking of proteins for degradation by hypothetical signals which require ATP [37], because results similar to those shown in Table 1 were obtained when the proteolysis *in vitro* was measured in extracts of cells incubated for 30 min with 10 mM-vanadate immediately after pulse-labelling (results not shown).

The reversibility of the vanadate effect, together with the observed increased degradation after removal of the inhibitor (Fig. 3), similar to the catch-up effect produced by some lysosomal inhibitors [38], suggests that the vanadate effect is due to the inhibition of the proteolytic system responsible for the degradation of short-lived proteins and that, in the presence of vanadate, short-lived proteins accumulate in their degradation site.

It has been reported in isolated hepatocytes that vanadate inhibits lysosomal proteinases [22,25], and inhibitory effects of vanadate on neutral ATP-dependent [23–27] and non-ATP-dependent [27] proteinases have also been described. In corroboration of this, we show here that this reagent inhibits some cysteine proteinases (lysosomal and non-lysosomal) of L-132 cells (Table 1), as well as the lysosomal degradation of long-lived proteins under step-down conditions and of endocytosed proteins (Tables 2 and 3), two processes believed to occur in lysosomes [28,29]. Most of the proteinases known to be inhibited by vanadate are ATP-dependent [23–27]. However, in extracts of cells the proteolysis was only slightly increased by ATP. Obviously, these results do not rule out the existence of ATP-dependent proteinases in exponentially growing cultured cells (i.e. although ATP does not increase proteolysis in rat liver homogenates, an ATP-stimulated proteinase from these homogenates has been isolated and characterized [39]). Further studies with the proteinase(s) purified from exponentially growing cultured cells and sensitive to vanadate should clarify this point.

The explanation for the increased degradation of long-lived proteins in the presence of vanadate (Figs. 1, 2 and 3) is not clear at this time. A similar increased degradation has been obtained in the presence of cytochalasin B, although no effect was observed on degradation of short-lived proteins [9]. We have confirmed these observations (cytochalasin B was used at a final concentration of 100 μg/ml).

The additive inhibitory effects of centrifugation and vanadate (Table 4) suggest that they affect different sites in the degradation of short-lived proteins. Indeed, this process may be totally inhibited by centrifugation and vanadate, because the remaining 1 % not affected by the two treatments could represent the contribution of long-lived proteins (whose degradation is not inhibited by these treatments [30]; the present paper) to the pool of short-lived proteins. The effect of vanadate on degradation of short-lived proteins appears to occur by inhibiting intracellular cysteine proteinases, whereas the effect of centrifugation remains to be clarified. Characterizing the vanadate-sensitive cell proteinase(s) or cell processes responsible for the observed effects should lead to a better understanding of the degradation of intracellular proteins in exponentially growing cultured cells. In conclusion, we show for the first time in exponentially growing cultured cells that vanadate inhibits degradation of short-lived proteins, whereas it increases the degradation of long-lived proteins, suggesting that their mechanism(s) of degradation are different.

We are particularly grateful to Professor Santiago Grisolía, whose help and discussions were invaluable. We also thank Dr. F. Thompson for carefully reading the manuscript, and A. Montaner and F. Ripoll for technical assistance. This work was supported by the FIS, U.S.–Spain Joint Committee for Scientific and Technological Cooperation, Institució Valenciana d'Estudis i Investigació, and by the CAICYT (grants 0574/84 and PB87-0585). F.A. is a Fellow of the Conselleria de Cultura, Educación y Ciencia de la Generalitat Valenciana.

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Received 3 May 1988/1 August 1988; accepted 7 October 1988