The reduction-oxidation status may influence the degradation of glyceraldehyde-3-phosphate dehydrogenase

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Received 7 July 1986

NADH and NADPH accelerate the 'in vitro' rate of proteolysis of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by elastase and other proteases, including lysosomal proteases. NAD^+ and $NADP^+$ have the opposite effect. Since there is a good correlation between proteolytic susceptibility of proteins and their 'in vivo' degradation rates, a possible role of the reduction-oxidation status in controlling the intracellular degradation of GAPDH is advanced.

Proteolysis *Pyridine nucleotide* *Redox status* *Glyceraldehyde-3-phosphate dehydrogenase* *Protease* *Intracellular degradation*

1. INTRODUCTION

There are large differences in the turnover rates of intracellular proteins and the turnover of individual enzymes may themselves vary widely under different conditions (review [1–4]). Therefore, specific mechanisms should be involved.

The half-lives of proteins may depend on their different susceptibilities to the proteolytic systems [5–8]. Since enzymes are flexible molecules which can have their susceptibility to proteolytic attack altered upon binding of substrates, cofactors and/or other ligands, to clarify the mechanism(s) which regulate(s) protein turnover it would seem logical to study individual well-selected proteins.

We have used GADPH (EC 1.2.1.12; D-glyceraldehyde 3-phosphate:NAD^+ oxidoreductase (phosphorylating)), because it is a cytosolic enzyme (thus minimizing problems arising from transfer of cytosolic precursors to other compartments (e.g. mitochondria)), it is a key enzyme catalyzing the single oxidative step in glycolysis and is very abundant, constituting about 20% and 10% of the soluble protein in yeast [9] and muscle [10], respectively. Selection of GAPDH was also based on the fact that its 'in vitro' inactivation mechanism had been extensively studied [5,11–13].

Abbreviations: β-NAGase, β-*N*-acetyl glucosaminidase; DTT, dithiothreitol; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

2. MATERIALS AND METHODS

GAPDH (from rabbit muscle) was purchased from Boehringer (Mannheim) as a crystal suspension and ammonium sulphate was removed [14]. Elastase (from porcine pancreas, 70 units/mg) and elastatinal (an inhibitor of elastase) were from Sigma (St. Louis, MO).

Lysosomes were prepared as in [15], suspended in 3 vols of 5 mM DTT and disrupted by freezing and thawing, 10 times.

Incubations were carried out at 37°C (final volume: 500 μl). Assays contained 0.5 mg

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00145793/86/$3.50

GAPDH, 0.1 M triethanolamine buffer, pH 7.6, 5 mM DTT, 1 mM EDTA, elastase or other protease (1 or 2% of GAPDH in terms of protein) and ligands (5 mM). At the times indicated, portions were taken and assayed for activity (diluted 10 times in 0.1 M triethanolamine buffer, pH 7.6, 5 mM DTT, 1 mM EDTA and 50 μM elastatinal) or subjected to SDS-PAGE [16]. In experiments with [^{14}C]GAPDH (final volume: 0.1 ml), portions were transferred to Whatman 3 MM filter paper discs and after washing the filters [17], radioactivity incorporated into protein was estimated by liquid scintillation counting.

GAPDH and β-NAGase activities were determined as in [18] and [19], respectively. GAPDH was labeled with [^{14}C]formaldehyde by reductive methylation [20]. The method of Bradford [21] was used to measure protein. All values represent means of three or more independent experiments with duplicated samples.

3. RESULTS AND DISCUSSION

Under the standard incubation conditions used here, GAPDH was stable per se but inactivated by elastase (fig.1). Addition of NADH enhanced the inactivation, whereas NAD^+ protected it. Of course, NADH also inactivated GAPDH in the absence of elastase [12]. Densitometric analysis of samples incubated as described and examined by SDS-PAGE (fig.2) indicated concomitant disappearance of the native subunit of M_r 36000 and loss of enzyme activity (proteolytic inactivation). When NAD^+ and NADH were used together and at the same concentrations (5 mM), the inactivation of GAPDH by elastase increased over that observed in the absence of pyridine nucleotides. Other experiments showed that $NADP^+$ protected GAPDH from proteolysis and that NADPH enhanced the proteolysis by elastase (fig.3), although $NADP^+$ was less effective than NAD^+. The observed effects of pyridine nucleotides on proteolysis of GAPDH with elastase were also apparent when the loss of trichloroacetic acid-soluble material from ^{14}C-labeled GAPDH was measured (not shown). None of these ligands had an effect on elastase activity (assayed with azoalbumin). Essentially the same results were obtained with other proteases (pronase, trypsin) and with broken

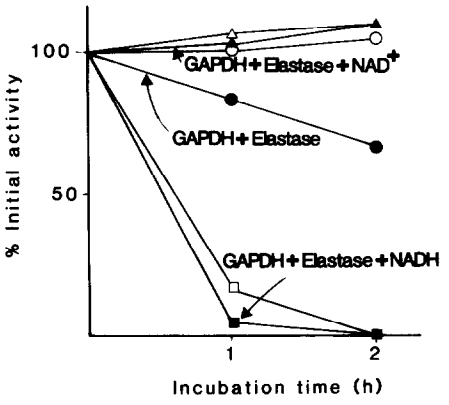

Fig.1. Effect of NAD^+ and NADH on the inactivation of GAPDH by elastase (1%, w/w, in terms of protein). Incubations were as described in section 2. (○) GAPDH, (●) GAPDH + elastase, (□) GAPDH + NADH, (■) GAPDH + elastase + NADH, (△) GAPDH + NAD^+, (▲) GAPDH + elastase + NAD^+.

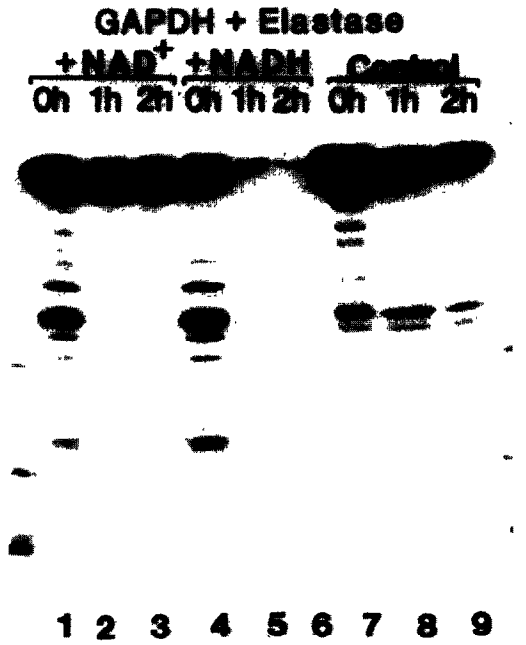

Fig.2. Effect of NAD^+ and NADH on the proteolysis of GAPDH by elastase. Same conditions from fig.1. The gel (12% acrylamide) was heavily loaded (40 μg GAPDH at 0 time) to show the effects more clearly. Lanes: 1–3, GAPDH + elastase + NAD^+, 0, 1 and 2 h incubation; 4–6, GAPDH + elastase + NADH, 0, 1 and 2 h incubation; 7–9, GAPDH + elastase (control), 0, 1 and 2 h incubation.

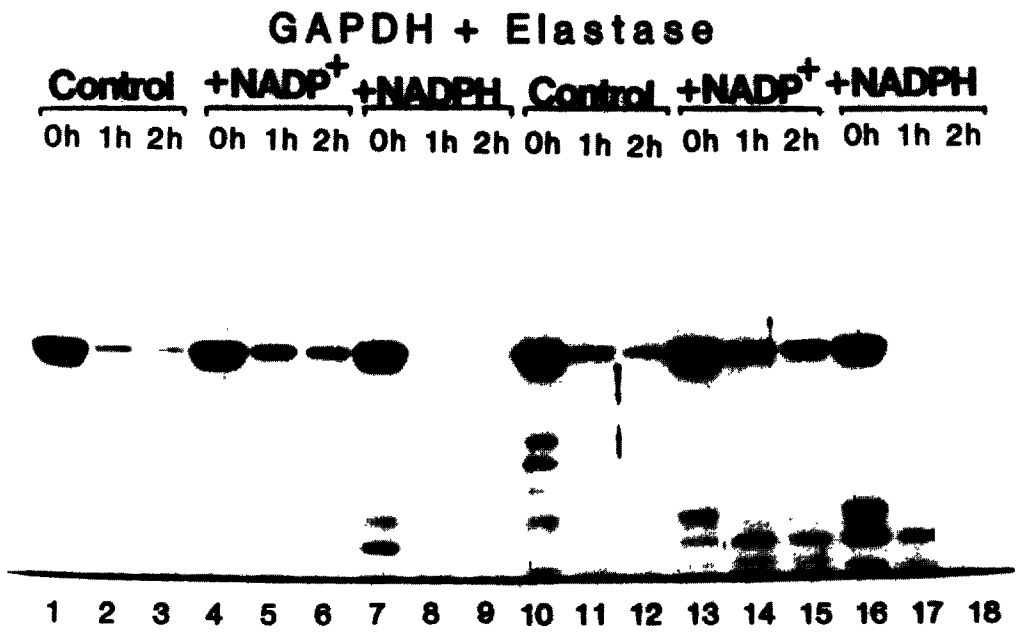

Fig.3. Effect of $NADP^+$ and NADPH on the proteolysis of GAPDH by elastase (2%, w/w). Two different experiments (lanes 1–9 and 10–18). Lanes: 1–3 and 10–12, GAPDH + elastase (control), 0, 1 and 2 h incubation; 4–6 and 13–15, GAPDH + elastase + $NADP^+$, 0, 1 and 2 h incubation; 7–9 and 16–18, GAPDH + elastase + NADPH.

lysosomes from rat liver (not shown and fig.4). It should be noted that a protective effect of NAD^+ against inactivation of GAPDH by lysates of rabbit liver lysosomes has already been reported [22]. Thus, the described effects of pyridine nucleotides are on the GAPDH molecule and not restricted to elastase.

It has been shown in rat diaphragm and atrium that when the cell is in a more catabolic status the redox couples are more oxidized [23], although, more recently, it has also been found that, in isolated skeletal muscle, general protein degradation can fall without a concomitant change in the redox status [24]. We now show here that the redox status could also affect the specific degradation of certain proteins, as exemplified here by GAPDH, in a direction opposite to that previously reported for total protein [23]. This is not unexpected because some degree of selectivity in protein turnover is needed to explain the different half-lives of intracellular proteins [1–4].

It seems, therefore, possible that the half-life of GAPDH is modulated by physiological fluctuations of the cytosolic redox status. By binding

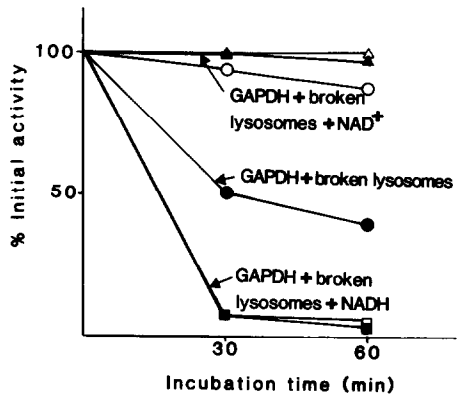

Fig.4. Effect of NAD^+ and NADH on the inactivation of GAPDH by broken lysosomes (0.4 units β-NAGase/mg GAPDH) from rat liver [15]. Incubations were carried out in 20 mM 2-[*N*-morpholino]ethanesulfonic acid, pH 5.5, 5 mM DTT. (○) GAPDH, (●) GAPDH + broken lysosomes, (□) GAPDH + NADH, (■) GAPDH + broken lysosomes + NADH, (△) GAPDH + NAD^+, (▲) GAPDH + broken lysosomes + NAD^+.

pyridine nucleotides GAPDH can change its conformation to expose or mask regions which make it more or less susceptible to the proteolytic systems, whatever they are, involved in the degradation of GAPDH. Since pyridine nucleotides are important components of the energy-transferring and oxidation-reduction reactions of the cell and the redox status varies with the metabolic situation of the cell, this is an attractive possibility. Microinjection of radioactive labeled GAPDH into cells and studying its degradation under different situations which modify the intracellular redox status will be the objectives of further investigation.

ACKNOWLEDGEMENTS

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. Supported in part by CAICYT, FISS, Comité Conjunto Hispano-Norteamericano and the IIC-KUMC International Molecular Cytology Program.

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