Volume 247, number 2, 197-200 FEB 07023 April 1989

Na^+, K^+-specific inhibition of protein and peptide hydrolyses by proteasomes from human hepatoma tissues

Jae Hong Seol, Sang Chul Park+, Doo Bong Ha, Chin Ha Chung, Keiji Tanaka* and Akira Ichihara*

Department of Zoology, College of Natural Sciences, +Department of Biochemistry, School of Medicine, Seoul National University, Seoul 151-742, South Korea and **Institute for Enzyme Research, Tokushima University, Tokushima 770, Japan*

Received 9 February 1989

Proteasomes were purified from human hepatoma tissues, and their sensitivities to Na^+ and K^+ were examined. At concentrations of 10 mM or more, these cations were found to inhibit completely polylysine-activated casein degradation by the purified proteasomes. They also strongly inhibited the hydrolyses of peptides, although to a lesser extent. On the other hand, they reversed the inhibitory and stimulatory effects of polylysine on the hydrolyses of Suc-Leu-Tyr-AMC and Cbz-Ala-Arg-Arg-MNA, respectively. These results suggest that Na^+ and/or K^+ may be involved in the regulation of intracellular protein breakdown by controlling the multicatalytic activity of proteasomes.

Proteasome; Proteolysis; Na^+ effect; K^+ effect; (Human hepatoma)

1. INTRODUCTION

Proteasomes, also called large multicatalytic protease complexes, are 20 S ring-shaped particles consisting of multiple, non-identical subunits with sizes ranging from 22 to 33 kDa [1,2]. They contain at least three distinct catalytic sites, that are specific for the hydrolyses of various proteins and hydrophobic, basic and acidic peptides and have neutral or alkaline pH optima [1,2]. These protease activities appear to be present in a latent form because purified proteasomes can be activated by polylysine, fatty acids or SDS [1,3,4].

This protease complex has been found in a variety of eukaryotic tissues and cells including human liver [1]. However, there has so far been no report on the purification and properties of the enzyme in malignant tissues of human. Tanaka et al. [1] have recently isolated and characterized in detail proteasomes from human liver. Therefore, we purified the protease complex from human hepatoma tissues as an attempt to compare the properties of the enzyme to those of normal liver proteasome. During its purification, we fortuitously found that the protease is sensitive to inhibition by low salt concentrations. In this study, we examined the effects of various monovalent and divalent cations on the multicatalytic activity of purified proteasomes. Two monovalent cations, Na^+ and K^+, were found to inhibit the activity particularly strongly, and the nature of this inhibition is discussed.

Correspondence address: C.H. Chung, Department of Zoology, College of Natural Sciences, Seoul National University, Seoul 151-742, Korea

2. MATERIALS AND METHODS

Proteolytic activity was assayed by measuring the conversion of [^{3}H]methyl-casein to materials soluble in 10% trichloroacetic acid [3,5]. Hydrolyses of peptides were assayed by fluorometric measurement of the releases of aminomethylcoumarin (AMC) and methoxynaphthylamide (MNA) from succinyl(Suc)-Leu-Tyr-AMC and carbobenzoxy(Cbz)-Ala-Arg-MNA [3,6].

Proteasomes were purified from human hepatoma tissue (70 g) by the procedure described by Tanaka et al. [3] but using hydroxyapatite column chromatography as the final step of purification. Neoplastic human liver tissue was obtained from

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

Seoul National University Hospital. [^{3}H]Methyl-casein was prepared as described previously [5].

3. RESULTS AND DISCUSSION

The purification of proteasomes from human hepatoma tissues was followed using Suc-Leu-Tyr-AMC as substrate. The elution profile of the enzyme complex from the hydroxyapatite column is shown in fig.1. Like proteasomes isolated from other sources, such as normal human or rat liver [1], the enzyme consisted of multiple subunits ranging in size between 22 and 33 kDa.

During the purification, we found that the recovery of peptidase activity increased about 2-fold after dialysis of the pooled fractions from ion-exchange columns against salt-free column buffer (not shown). Therefore, we tested if NaCl, which was used for formation of ion gradients in columns, inhibited peptide cleavage by the proteasomes. As shown in fig.2, NaCl at as low a concentration as 10 mM caused more than 50% inhibi-

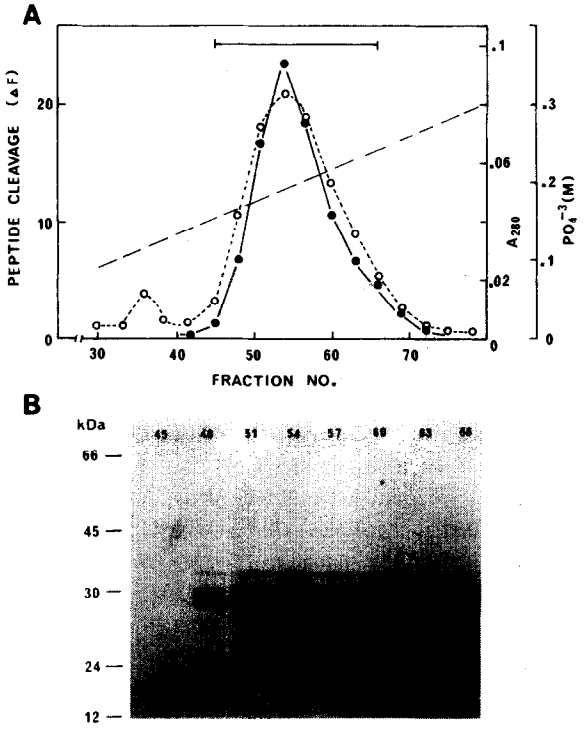

Fig.1. Elution profile of proteasomes from a hydroxyapatite column. Proteasomes were purified as described by Tanaka et al. [3] but using Suc-Leu-Tyr-AMC as a substrate and neoplastic human liver tissue as the source of enzyme. (A) Chromatography on a hydroxyapatite column (1 × 5.5 cm) was carried out at a flow rate of 14 ml/h. (○) Protein; (●) peptidase activity; (– – –) phosphate gradient. (B) Aliquots of the fractions under the bar in (A) were subjected to electrophoresis in a 12.5% (w/v) polyacrylamide gel containing SDS. The yield of purified proteasomes from 70 g of tissues was 1.4 mg.

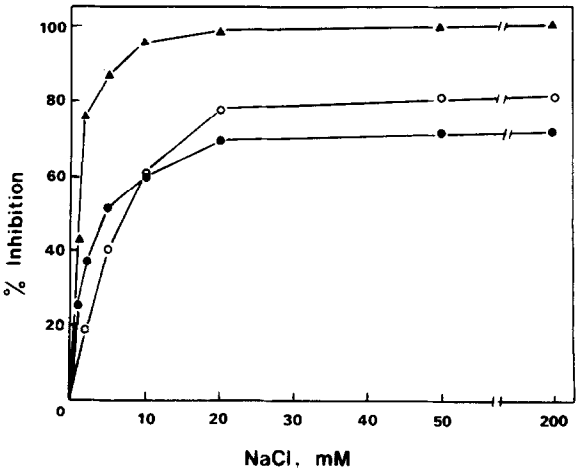

Fig.2. Effects of increasing concentration of NaCl on protein and peptides hydrolyses by proteasomes. Protein and peptides were incubated for 30 min at 37°C with 0.5 and 0.1 μg of purified proteasomes, respectively. The substrates used were casein (▲), Suc-Leu-Tyr-AMC (●), and Cbz-Ala-Arg-Arg-MNA (○).

Table 1

Effects of various cations on the hydrolyses of protein and peptides by proteasomes

Addition	% Relative hydrolysis		
	Suc-Leu-Tyr-AMC	Cbz-Ala-Arg-Arg-MNA	Casein
None	100	100	100
NaCl	40	45	12
KCl	59	51	22
LiCl	81	123	115
CsCl	104	85	111
$MgCl_2$	105	93	108
$CaCl_2$	138	99	16
$MnCl_2$	154	51	30

Reaction mixtures contained 0.1 μg of the purified enzyme and 10 mM cations for peptide hydrolysis and 0.5 μg of the protease and 2 mM cations for casein degradation. Incubations were performed at 37°C for 30 min. Casein hydrolysis was measured in the presence of 0.1 mg/ml of polylysine, as little or no activity can be seen without the agent

Table 2

Effect of polylysine on the hydrolyses of peptides in the presence and absence of Na^+

Peptide (0.1 mM)	Polylysine (0.1 mg/ml)	% Relative activity		Ratio (+NaCl/−NaCl)
		−NaCl	+NaCl	
Suc-Leu-Tyr-AMC	−	100	36	0.4
	+	18	107	5.9
Cbz-Ala-Arg-Arg-MNA	−	100	52	0.5
	+	291	232	0.8

Proteasomes were treated with polylysine in the presence and absence of 10 mM NaCl. Incubations were carried out as described for table 1. The activity without additions was expressed as 100%. Similar results were obtained on addition of polylysine with and without K^+

tion of the hydrolyses of both Suc-Leu-Tyr-AMC and Cbz-Ala-Arg-Arg-MNA. However, even high NaCl concentrations of up to 0.2 M did not cause complete inhibition. Similar findings had been reported by Orlowski and his colleagues for the activity of a neutral endopeptidase from bovine pituitary on different substrates [7–9]. Polylysine-activated casein degradation was more sensitive than peptide cleavage to NaCl and was almost completely inhibited by 10 mM NaCl. Assuming that eukaryotic cells contain certain polylysine-like proteins (i.e. highly basic proteins), proteasomes should function in autolysis of intracellular proteins. Thus, Na^+, K^+ or both may prevent the unnecessary breakdown of cell proteins by this latent enzyme.

We then examined if other cations are also capable of inhibiting the hydrolyses of protein and peptide by proteasomes. Table 1 shows that K^+caused similar effects of inhibition of the two activities to those of Na^+. Little or no inhibition was observed with other monovalent cations tested. The hydrolysis of casein was also sensitive to inhibition by Ca^{2+} and Mn^{2+}, but not by Mg^{2+}, and Mn^{2+} also inhibited the cleavage of Cbz-Ala-Arg-Arg-MNA. These inhibitory effects of divalent cations may not, however, be physiologically significant, as divalent cations are known to be present in cells at only micromolar levels. After treatment with inhibitory cations, dialysis resulted in full recovery of the enzyme activities (data not shown), indicating that the inhibition was reversible.

The degradation of casein by the purified proteasome can only be seen in the presence of polylysine. Therefore, the effect of Na^+ on peptide hydrolysis in the presence and absence of polylysine was also examined. The cleavage of Suc-Leu-Tyr-AMC was markedly inhibited by polylysine, but addition of Na^+ with polylysine restored the activity to the same level as in the absence of addition (table 2). In contrast, polylysine stimulated the cleavage of Cbz-Ala-Arg-Arg-MNA about 3-fold and somewhat reduced the inhibitory effect of Na^+. Similar results were obtained with proteasomes isolated from normal human liver (data not shown), indicating that both structural and catalytic properties of the enzyme from the malignant tissues are identical to those of normal one. These results suggest that Na^+ and/or K^+ may control the multicatalytic activity of proteasomes and thereby regulate the intracellular hydrolysis of proteins and peptides. However, an additional mechanism seems to be necessary for activation of this latent enzyme in vivo, because physiological concentrations of the cations tend to cause complete inhibition of the enzyme activity for breakdown of proteins.

Acknowledgements: This work was supported in part by grants from Korea Science and Engineering Foundation and Ministry of Education to C.H.C.

REFERENCES

[1] Tanaka, K., Yoshimura, T., Kumatori, A., Ichihara, A., Ikai, A., Nishigai, M., Kameyama, K. and Takagi, T. (1988) J. Biol. Chem. 263, 16209–16217.

[2] Tanaka, K., Yoshimura, T., Ichihara, A., Ikai, A., Nishigai, M., Morimoto, Y., Sato, M., Tanaka, N., Katsube, Y., Kameyama, K. and Tanagi, T. (1988) J. Mol. Biol. 203, 985–996.

[3] Tanaka, K., Ii, K., Ichihara, A., Waxman, L. and Goldberg, A.L. (1986) J. Biol. Chem. 261, 15197–15203.
[4] Dahlmann, B., Rutschmann, M., Kuehn, L. and Reinauer, H. (1985) Biochem. J. 228, 171–177.
[5] Hwang, B.J., Woo, K.M., Goldberg, A.L. and Chung, C.H. (1988) J. Biol. Chem. 263, 8727–8734.
[6] Woo, K.M., Chung, W.J., Goldberg, A.L. and Chung, C.H. (1989) J. Biol. Chem. 264, in press.
[7] Wilks, S., Pearce, S. and Orlowski, M. (1979) Life Sci. 24, 457–464.
[8] Wilks, S. and Orlowski, M. (1980) J. Neurochem. 35, 1172–1182.
[9] Wilks, S. and Orlowski, M. (1983) J. Neurochem. 40, 842–849.