Human Cathepsin O2, a Matrix Protein-degrading Cysteine Protease Expressed in Osteoclasts

FUNCTIONAL EXPRESSION OF HUMAN CATHEPSIN O2 IN SPODOPTERA FRUGIPERDA AND CHARACTERIZATION OF THE ENZYME*

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Cathepsin O2, a human cysteine protease predominately present in osteoclasts, has been functionally expressed in Spodoptera frugiperda Sf9 cells using the Autographa californica nuclear polyhedrosis virus. Following in vitro activation at pH 4.0 with pepsin, active enzyme with an apparent molecular weight of 29,000 was obtained. N-terminal sequencing revealed the typical processing site for cysteine proteinases of the papain family with a proline in the position adjacent to the N-terminal alanine residue. The S$_{3}$P$_{2}$ subsite specificity of human cathepsin O2 is similar to cathepsin S but distinguished from cathepsins L and B. Similar to cathepsin S, cathepsin O2 is characterized by a bell-shaped pH activity profile and is stable at pH 6.5 for 30 min at 37 °C. Cathepsin O2 is further distinguished by its potent collagenolytic activity against Type I collagen between pH 5 and 6, and elastinolytic activity against insoluble elastin at pH 7.0.

Its capacity to efficiently degrade Type I collagen and its high expression in osteoclasts suggest that cathepsin O2 may play a major role in human osteoclastic bone resorption.

Bone tissue is constantly undergoing a process of formation and resorption. Osteoclasts are cells responsible for the bone resorbing process. Bone resorption includes demineralization and degradation of extracellular matrix proteins (Delaisse and Vaes, 1992). Type I collagen constitutes 95% of the organic matrix (Kranien and Simon, 1994). In addition to the interstitial collagenase, the lysosomal cysteine proteinases cathepsins B and L are thought to be involved in osteoclastic bone resorption (Delaisse and Vaes, 1992). Both enzymes are present in the lysosomes as well as in the acidified extracellular resorption lacuna of the osteoclasts (Goto et al., 1993), and both proteases display the in vitro ability to degrade collagen Type I at acidic pH (Maciewicz et al., 1987; Delaisse et al., 1991). Cysteine proteinase inhibitors, such as E-64 and leupeptin, have been shown to prevent osteoclastic bone resorption (Delaisse et al., 1987; Everts et al., 1988). Cathepsin L is considered to be one of the main proteinases involved in collagen degradation in bone (Maciewicz and Etherington 1988; Kakegawa et al., 1993). It is thought that the collagenolytic action of cysteine proteinases is exerted preferentially in the most acidic part of the bone resorption lacuna close to the ruffled border at a pH around 3.5 or 4.5, whereas the zinc-containing collagenases are more active in the neutral environment at the interface between the demineralized and mineralized matrix (Delaisse and Vaes, 1992). Besides cathepsins L and B, a variety of cathepsin L- and B-like activities may participate in collagenolytic bone degradation. Page et al. (1992) isolated multiple forms of cathepsin B from osteoclastomas. These have an acidic pH optimum and the ability to degrade soluble and insoluble Type I collagen. Delaisse et al. (1991) identified a 70-kDa thiol-dependent protease in bone tissue, which is also capable of degrading Type I collagen.

More recently, a cDNA encoding a novel human cysteine proteinase protease was cloned independently by several groups (Shi et al., 1995; Inaoka et al., 1995; Brömmel and Okamoto, 1995) and named cathepsin O, cathepsin K, and cathepsin O2. All three sequences are identical and represent the human equivalent of the OC2 gene cloned from rabbit osteoclasts (Tezuka et al., 1994). This novel human cathepsin is highly expressed in osteoclasts and displays an approximate 10-fold higher expression at the message level when compared with cathepsin L (Brömmel and Okamoto, 1995). Saneshige et al. (1995) could show that cathepsin O2(K) is regulated at the transcriptional level by retinoic acid in osteoclasts. Retinoic acid is a potent regulator and enhances the proliferation and differentiation of many types of cells including osteoclasts. Shi et al. (1995) demonstrated an endopeptidase activity of this enzyme against fibrinogen at acidic pH when overexpressed in COS-7 cells. However, no data have been published describing the enzymatic properties of this protease and demonstrating its activity toward extracellular matrix proteins such as collagen and elastin. In this publication, we describe for the first time the functional expression of recombinant cathepsin O2, its purification, enzymology, and inhibitor profile as well as its ability to efficiently degrade matrix proteins.

EXPERIMENTAL PROCEDURES

Materials—Z-LLR-MCA, Z-VR-MCA, and Z-RR-MCA were obtained from Aminotech (Canada). Z-FR-MCA, Z-VVR-MCA, and Z-LLR-MCA were synthesized as described elsewhere (Brömmel et al., 1989, 1993). Type I collagen was purchased from U. S. Biochemical Corp. Z-F-CHN$_{2}$ and Z-FA-CHN$_{2}$ were obtained from Bachem Bioscience, Inc., Switzerland.

Construction of Transfer Vector and Expression—Using the polymerase chain reaction, a BglII site at the 5’-end of the preregion of human cathepsin O2 and a BamHI site at the 3’-end of the mature region of the enzyme were placed. The amplification reaction was carried out with the Pfu DNA polymerase (Stratagene, La Jolla, CA). The 1.1-kb fragment was inserted into the BglII and BamHI site of the pVL392 transfer vector (PharMingen, San Diego, CA). Recombinant baculovirus 

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The abbreviations used are: Z-, benzyloxycarbonyl; -MCA, 4-methyl-7-coumarylamide; E-64, L-3-carboxy-trans-2,3-epoxycycropropyl-leucylamido(4-guanidino)butane; CHN$_{2}$, diazomethane.
was generated by homologous recombination following cotransfection of the baculovirus transfer vector and linearized AcNPV genomic DNA into SF9 cells (PharMingen, San Diego, CA). Pure virus (AcNPV[CO2]) was obtained by plaque purification. SF9 cells were grown in 1 liter of S9001 media (Life Technologies, Inc.) to a density of 2 × 10^6 cells/ml and infected at a multiplicity of infection of 1. Total cell number and cellular and secreted activity of cathepsin O2 were monitored every 24 h. After 3.5 days, the cells were harvested at a cell density of 2.5 × 10^9/ml.

Activation, Purification, and N-Terminal Sequencing—The SF9 cells were harvested from the production media by centrifugation at 2,000 × g and were lysed in a Dounce homogenizer. The cell lysate containing inactive cathepsin O2 precursor was brought up to 100 ml with 100 mM sodium acetate buffer, pH 7.0 with 2 M Tris base, clarified by centrifugation at 10,000 × g. NH2-terminal sequencing was carried out by automated Edman degradation.

To determine the pH effect on protease activity, the pH stability of cathepsins O2, S, and L was studied at three different pH values. Recombinant human cathepsins O2, S, and L were incubated at 37°C in 100 mM sodium acetate buffer, pH 5.5, in 100 mM potassium phosphate buffer, pH 6.5, and in 100 mM Tris-HCl, pH 7.5, containing 1 mM dithiothreitol and 2.5 mM EDTA. Incubating at pH 6.5 for 0.5, 1, 2, and 4 h, the activity remaining was determined using 5 M Z-FR-MCA for cathepsin O2 (100 mM potassium phosphate buffer, pH 6.5) and cathepsin L (100 mM sodium acetate buffer, pH 5.5) and 5 M Z-VVR-MCA for cathepsin S (100 mM potassium phosphate buffer, pH 6.5).

Results and Discussion

Expression and Activation of the Precursor of Human Cathepsin O2—SF9 cells infected with AcNPV[preproCatO2] were harvested 84 h postinfection. The majority of immunoreactive material of about 43 kDa was found within the infected cells. In

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contrast to the single product of 43 kDa in the culture medium, an additional slight band of 44 kDa was detected in the cellular extract (data not shown). We assume that the higher molecular weight band represents unprocessed proprocathepsin O2, whereas the 43-kDa protein is proenzyme. No activity was observed immediately after lysis of the cells nor during autoactivating conditions at 40 °C between pH 4.0 and 4.5 in the presence of dithiothreitol using the synthetic substrate Z-FR-MCA at pH 7.5. The increase of an E-64 inhibitable activity under autoactivating conditions and measured at pH 5.5 was mediated by a 36 kDa (Fig. 1). In parallel with this process an increase of E-64 inhibitable activity measured at pH 7.5 is observed.

Purification and N-terminal Sequencing—Human cathepsin O2 was purified to electrophoretic homogeneity using hydrophobic chromatography (butyl-Sepharose 4 Fast Flow) and ion-exchange chromatography (Mono S) (Fig. 2). The average yield of a 1-liter Sf9 cell culture (~2 × 10⁸ cells) was approximately 2 mg of purified enzyme (Table I).

The purified enzyme is a single-chain enzyme and exhibits an apparent molecular mass of 29 kDa in a 4–20% Tris/glycine SDS gel under reducing conditions. Treatment with endoglycosidases H and F as well as N-glycosidase F did not result in a shift in the molecular weight, which implies that the protease is not glycosylated (data not shown). Human cathepsin O2 has two potential glycosylation sites in its mature sequence (Brömme and Okamoto, 1995). However, both sites have either a proline residue adjacent to the asparagine or to the threonine, so that their use is unlikely (Gavel and von Heijne, 1990). Additionally, cathepsin O2 contains one putative glycosylation site in the propeptide close to the processing site between the mature enzyme and the propeptide. Again, no shift in molecular weight of the proenzyme was observed after overnight treatment with endoglycosidases H and F as well as N-glycosidase F.

N-terminal sequencing of the mature protease processed in presence of pepsin revealed the typical processing site described for cysteine proteases of the papain family with a proline adjacent to the N-terminal alanine (NH₂-APDS-)

![Image](399x639 to 470x732)

**Fig. 1. Maturation of procathepsin O2 with pepsin.** Aliquots of the culture supernatant containing procathepsin O2 were incubated with pepsin (0.4 mg/ml) at 40 °C in 100 mM sodium acetate buffer, pH 4.0. The incubation was stopped by adding sample buffer. The times of digestion are as indicated. Molecular mass standards (kDa) are indicated in the right margin.

**Fig. 2. SDS-PAGE of purified recombinant human cathepsin O2 (Coomassie Blue staining).** Lane 1, crude Sf9 fraction; lane 2, after passage through n-butyl fast Flow; lane 3, after passage through Mono S. Molecular mass standards are indicated in the right lane.

| Assay               | Total protein mg | Total activity µmol/min | Specific activity µmol/mg/min | Purification factor Yield % |
|---------------------|-----------------|------------------------|------------------------------|----------------------------|
| Crude 2.5 M (NH₄)₂SO₄ | 800             | 1,753                  | 2.2                          | 1                          | 100                      |
| Soluble fraction    | 276             | 1,412                  | 5.1                          | 2.3                        | 81                       |
| Butyl-Sepharose 4   | 2.9             | 1,143                  | 394                          | 179                        | 65                       |
| Mono S              | 1.1             | 512                    | 465                          | 211                        | 29                       |

*After activation with pepsin.*

**Table I**

Purification of recombinant human cathepsin O2 from 1 liter of Sf9 culture

VYDVKKGYVTVPKN (Rowan et al., 1992). Since a cleavage by pepsin after arginine (position –1) is very unlikely, it is possible that this site was generated after an initial cleavage by pepsin in the propeptide region followed by an aminopeptidase activity present in the Sf9 cell extract or by an active intermediate of cathepsin O2 generated by pepsin. Most aminopeptidases will stop at an alanine residue (position 1 of the mature enzyme sequence) due to the subsequent proline residue. This mechanism of processing involving aminopeptidases is also discussed for procathepsin B (Rowan et al., 1992).

In contrast, autocatalytically activated cysteine proteases frequently have at their processing site an N-terminal extension of 3–6 amino acids from the propeptide (Brömme et al., 1993). No activation of the precursor was observed by addition of purified active cathepsin O2 at pH 4.5 (data not shown), indicating that neither an initial cis nor trans autoactivation of cathepsin O2 within the lysosomes is likely. This contrasts related cysteine proteases such as papain and cathepsin S, which exhibit a potential autocatalytic activation pathway (Vernet et al., 1990; Brömme et al., 1993).

The activating enzyme of cathepsin O2 within the osteoclast could be the aspartyl protease cathepsin D, which is present in osteoclastic lysosomes but secreted at low levels into the resorption lacuna (Goto et al., 1993).

**S₂P₂ Subsite Specificity of Recombinant Human Cathepsin O2**—The S₂P₂ specificity of human cathepsin O2 was characterized using synthetic substrates of the type Z-XR-MCA with X equal to F, L, V, or R. The S₂ substite pocket of cysteine proteases is structurally well defined and determines the primary specificity of this protease class. For example, cathepsin B contains a glutamate (Glu-245) residue at the bottom of the S₂ subsite pocket, which favors the binding of basic residues like arginine. This glutamate residue is replaced by neutral residues in all other known human cathepsins resulting in a very low hydrolysis rate of the Z-RR-MCA substrate. Cathepsin O2 contains a leucine residue in position 205 which makes Z-RR-MCA a very poor substrate (Fig. 3). The specificity of cathepsin O2 toward P₂ residues resembles that of cathepsin S. Both enzymes prefer a leucine over a phenylalanine in this position, while cathepsin L is characterized by an inverse specificity (Table II, Fig. 3). Valine in position P₂ is relatively well

D. Brömme, unpublished results.
accepted by cathepsin O2, whereas the presence of this \( \beta \)-branched residue in \( P_2 \) results in a poor substrate for cathepsins L, S, and B.

The catalytic efficiency (\( k_{\text{cat}}/K_m \)) of cathepsin O2 toward dipeptide substrates is comparable with that of cathepsins S and B but is approximately 1 order of magnitude lower than that of cathepsin L. Interestingly, the \( k_{\text{cat}} \) values for cathepsin O2 are comparable with those determined for cathepsin L. The \( K_m \) value reflects to some extent the affinity of the substrates for the proteases. This trend is even more obvious for the tripeptide substrate, Z-LLR-MCA, which displays a \( K_m \) value as low as \( 4 \times 10^{-7} \) M (Table II). However, in contrast to cathepsins S and L, the \( k_{\text{cat}} \) values are almost 2 orders of magnitude lower for cathepsin O2.

**pH Activity Profile and pH Stability of Human Recombinant Cathepsin O2**—Profiles of \( pH \) activity are sensitive measures of enzymatic functional and structural integrity. A comparison of \( pH \) profiles from different but related proteases reveals differences in intrinsic activity and stability of these proteases. Human cathepsin O2 displays a bell-shaped \( pH \) profile with flanking \( pK \) values of 4.0 and 8.13 (Table III; Fig. 4). Its \( pH \) optimum is between 6.0 and 6.5 and comparable with that observed for cathepsin S (Brömmel et al., 1991). The width of the \( pH \) profile, which mirrors the stability of the ion-pair (Menard et al., 1991), is 4.15 for cathepsin O2 but only 3.35 for cathepsin S (Brömmel et al., 1993). This parameter for human cathepsin O2 is more similar to that observed for the very stable papain, which displays a profile width of 3.91 (Khouri et al., 1991).

Human cathepsin O2 is more stable than cathepsin L at slightly acidic to neutral \( pH \) values but less stable than cathepsin S (Table IV). Approximately 50% of the cathepsin O2 activity remains after 1 h at \( 37^\circ \)C and \( pH \) 6.5, whereas essentially no cathepsin L activity could be observed under these conditions.

However, it must be considered that the \( pH \) stability was determined without substrate protection, which usually increases the \( pH \) stability. In the \( [3H] \)elastin degradation assay with cathepsin O2, an increase of solubilized \( [3H] \) fragments was still observed after 2 h at \( pH \) 7.0.

**Inhibitor Profile of Human Cathepsin O2**—Human cathepsin O2 displays a typical inhibitor profile of a cysteine protease. It is inhibited by cysteine protease inhibitors and by inhibitors of both cysteine and serine proteases (Table V). At concentrations above 0.1 \( \mu M \), peptide aldehydes, diazomethanes, E-64, and chicken cystatin completely inhibit enzyme activity. On the other hand, specific serine and aspartic protease inhibitors do not affect enzyme activity. No effect of EDTA at a concentration of 4 mM was observed on the activity of cathepsin O2. At higher concentrations (>5 mM) a partial nonspecific inhibition was observed.

**Degradation of Type I Collagen and Elastin by Human Cathepsin O2**—The elastinolytic activity of human cathepsin O2 was measured at \( pH \) 4.5, 5.5, and 7.0 against insoluble \( [3H] \)elastin. Maximal activity was observed at \( pH \) 5.5. Cathepsin O2 displays a profile width of 4 mM was observed on the activity of cathepsin O2. At higher concentrations (>5 mM) a partial nonspecific inhibition was observed.
TABLE IV
pH stability at 37 °C of recombinant human cathepsin O2 in comparison with recombinant human cathepsin S and rat cathepsin L

| Protease     | Incubation time (h) | Residual activity at pH 5.5 | Residual activity at pH 6.5 | Residual activity at pH 7.5 |
|--------------|---------------------|----------------------------|----------------------------|----------------------------|
| Cathepsin O2 | 0.5                 | 91%                        | 85%                        | 11%                        |
|              | 1                   | 88%                        | 49%                        | 0%                         |
|              | 2                   | 70%                        | 22%                        | 0%                         |
|              | 4                   | 52%                        | 15%                        | 0%                         |
| Cathepsin S  | 0.5                 | 100%                       | 100%                       | 91%                        |
|              | 1                   | 95%                        | 100%                       | 72%                        |
|              | 2                   | 92%                        | 94%                        | 61%                        |
|              | 4                   | 83%                        | 71%                        | 60%                        |
| Cathepsin L  | 0.5                 | 87%                        | 12%                        | 0%                         |
|              | 1                   | 78%                        | 3%                         | 0%                         |
|              | 2                   | 71%                        | 0%                         | 0%                         |
|              | 4                   | 51%                        | 0%                         | 0%                         |

TABLE V
Inhibitor profile of recombinant human cathepsin O2

| Inhibitor               | Concentration (mM) | Percent inhibition |
|-------------------------|--------------------|--------------------|
| Serine protease inhibitors |                   |                    |
| PMSF                     | 1 mM               | 0%                 |
| Pepablock                | 0.2 mM             | 0%                 |
| DCI                      | 0.1 mM             | 0%                 |
| Serine/cysteine protease inhibitors |       |                    |
| Leupeptin                | 0.05 µM            | 85%                |
| Chymostatin              | 0.05 µM            | 64%                |
| Calpeptin                | 0.1 µM             | 100%               |
| Aspartate protease inhibitor |                  |                    |
| Pepstatin                | 0.1 µM             | 0%                 |
| Metallo-protease inhibitor |                  |                    |
| EDTA                     | 4 mM               | 0%                 |
| Cysteine protease inhibitor |                |                    |
| Iodoacetate              | 50 µM              | 60%                |
| Z-FF-CHN2                | 0.1 µM             | 90%                |
| Z-FA-CHN2                | 0.1 µM             | 100%               |
| E-64                     | 0.1 µM             | 100%               |
| Chicken cystatin         | 0.1 µM             | 100%               |

FIG. 5. Elastolytic activity of recombinant human cathepsin O2 at pH 4.5, 5.5, and 7.0 in comparison to cathepsins S and L and pancreatic elastase (substrate, 3H-labeled insoluble elastin).

Cathepsin L also cleaves in the telopeptide region, but essentially no small molecular weight fragments were detected under the conditions used. The effective pH range for the collagenolytic activity of cathepsin L is more acidic when compared with that observed for cathepsin O2 (between pH 4.0 and 5.5). Cathepsin S seems to reveal only a very weak collagenolytic activity. In contrast, tissue collagenases cleave the monomers into 3/4 and 1/4 fragments (Gross and Nagai, 1965). No degradation of Type I collagen was observed with trypsin at equal enzyme concentration compared with cathepsin O2, showing that the integrity of the triple helix of the collagen used was not impaired (data not shown).

In addition to its collagenase activity, cathepsin O2 displays a powerful gelatinase activity. At 0.1 nM concentration of the enzyme, denatured collagen is totally degraded within 30 min within a pH range of 5.0–7.0. In contrast, cathepsin L displays its gelatinase activity only in the pH range between 4.5 and 5.5 (Fig. 6B). Cathepsin S is active between pH 4.0 and 7.0, but it displays a significantly weaker activity than that of cathepsins O2 and L.

Immunohistochemical Localization of Human Cathepsin O2 in Osteoclastoma Tissue—Expression of cathepsin O2 was previously detected by Northern blot analysis at high levels in osteoclastomas (Brömme and Okamoto, 1995). Immunostaining of an osteoclastoma revealed specific staining of multinucleated osteoclasts, whereas stromal cells displayed only a weak immunoreactivity (Fig. 7).

The Potential Role of Cathepsin O2 in Osteoclastic Bone Resorption—Current data suggest that osteoclastic bone resorption is mostly linked to the activity of cathepsins L and B (Everts et al., 1992; Delaisse and Vaes, 1992; Kakegawa et al., 1993), but the results presented here suggest human cathepsin O2 as a potential key player in bone remodelling. Message levels in human osteoclastoma preparations (Brömme and Okamoto, 1995) exhibit a manyfold higher level of expression of cathepsin O2 than cathepsin L. Immunohistochemical staining of multinucleated osteoclasts in osteoclastoma sections (Fig. 7)
as well as osteoclasts in prenatal human bones confirm the expression of the protease at the protein level. Also, demonstrated for the first time, cathepsin O2 is a highly active cysteine protease that is capable of hydrolyzing extracellular matrix proteins such as collagen and elastin. Type I collagen, the major structural protein component in bone, is efficiently hydrolyzed at pH values above 5.5 by cathepsin O2, a pH region where only a low or no activity is observed for cathepsin L.

The pH value in the subosteoclastic resorption zone underneath the ruffled border is approximately between pH 3.5 and 4.5 (Fallon, 1984; Silver et al., 1988). However, it can be assumed that in the lower part of the resorption lacuna, at the interface between the demineralized and mineralized matrix, a pH gradient toward a neutral pH value exists due to the buffering capacity of dissolved bone salts. This area of the resorption lacuna is the proposed compartment for the action of interstitial collagenase, which is only active at slightly acidic to slightly alkaline pH (Delaisse and Vaes, 1992). The results presented for the pH stability, pH activity profile as well as the in vitro collagenase activity of cathepsin O2, suggest that this novel cysteine protease is active at the site where interstitial collagenase is active and that both activities may act in a concerted manner on Type I collagen. Besides cathepsin S (Brömme et al., 1993), cathepsin O2 is the second known human papain-like cysteine protease that displays a bell-shaped pH profile with a pH optimum at 6.0. Cathepsin L, due to its high instability at neutral pH (Turk et al., 1993) is excluded from this site and may be involved in a later degradation stage.

\(^4\) A. Rinne and D. Brömme, unpublished data.
of collagen in the more acidified microenvironment of the resorption lacuna. Demonstration of cathepsin O2 secretion into the bone resorption lacuna will need to be achieved in order to verify this hypothesis.

In addition, cathepsin O2 is distinguished by a very potent gelatinase activity in the pH range between 5 and 7, whereas the gelatinase activity of cathepsin L is limited to the pH range between 4 and 5.5. Consequently, a rapid degradation of the collagen cleavage products released by the interstitial collagenase is possible in the pH micro environment of the tissue collagenase.

Studies with cysteine protease inhibitors, E-64, leupeptin, and diazo methanes, clearly demonstrate that cysteine protease inhibitors are capable of inhibiting bone resorption (Delaisse et al., 1980, 1987; Debari et al., 1995). Since all three types of inhibitors are very effective on cathepsin O2, it is likely that these inhibition studies reflect to a large extent the inhibition of cathepsin O2.

In conclusion, cathepsin O2, is highly expressed in osteoclasts. Cathepsin O2 is distinguished from classical human cysteine proteases like cathepsins L and B by its increased stability at neutral pH as well as its ability to efficiently hydrolyze Type I collagen and elastin at pH values above 6.0. The expression of cathepsin O2 protein in osteoclasts as well as its enzymatic properties imply a central role in normal bone remodelling as well as in pathological processes, such as osteoarthritis, osteoporosis, and multiple myeloma osteoclastomas. The design of potent cathepsin O2 inhibitors could be an important contribution in the efforts to arrest these pathological processes.

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REFERENCES

Banda, M. J., Werb, Z., and McDerrow, J. H. (1987) Methods Enzymol. 144, 288–305
Barrett, A. J., and Kirschke, H. (1981) Methods Enzymol. 80, 535–561
Brömmel, D., and Okamoto, K. (1989) Biol. Chem. Hoppe-Seyler 367, 379–384
Brömmel, D., Steinitz, A., Friebe, S., Fittkau, S., Wiederanders, B., and Kirschke, H. (1989) Biochem. J. 264, 475–481
Brömmel, D., Bonneau, P. R., Ladanche, P., Wiederanders, B., Kirschke, H., Peters, C., Thomas, D. Y., Storer, A. C., and Vernet, T. (1993) J. Biol. Chem. 268, 4832–4838
Cattoretti, G., Becker, M. H., Key, G., Duchrow, M., Schüter, C., Galle, J., and Gerdes, J. (1992) J. Pathol. 169, 357–363
Debari, K., Sasaki, T., Udegawa, N., and Rifkin, B. R. (1995) Calcif. Tissue Int. 56, 566–570
Delaisse, J.-M., and Vaes, G. (1992) in Biology and Physiology of the Osteoclast (Rifkin, B. R., and Gay, C. V., eds) pp. 289–314. CRC Press, Boca Raton, FL
Delaisse, J.-M., Boyde, A., Macanouchie, E., Ali, N. N., Sear, C. H. J., Eedkhouy, Y., Vaes, G., and Jones, S. J. (1987) Bone 8, 305–313
Delaisse, J.-M., Ledent, P., and Vaes, G. (1991) Biochem. J. 279, 167–174
Everts, V., Beertsen, W., and Schröder, R. (1988) Calcif. Tissue Int. 43, 172–178
Everts, V., Delaisse, J. M., Korp, W., Niehof, A., Vaes, G., and Beertsen, W. (1992) J. Cell. Physiol. 150, 221–231
Falcon, M. D. (1994) in Endocrine Control of Bone and Calcium Metabolism (Cohn, D. V., Fujita, T., Potts, T. J., Jr., and Talmage, R. V., eds) p. 144. Elsevier, Amsterdam
Gavel, Y., and von Hentije, G. (1990) Protein Eng. 3, 442–443
Goto, T., Tsukuba, T., Kiyoshima, T., Nishimura, Y., Kato, K., Yamamoto, K., and Tanaka, T. (1993) Histochimistry 99, 411–414
Gross, J., and Nagai, Y. (1965) Proc. Natl. Acad. Sci. U.S.A. 54, 1197–1204
Inoua, T., Bille, G., Ishibashi, O., Tezuka, K., Kumeaga, M., and Kokubo, T. (1995) Biochem. Biophys. Res. Commun. 206, 89–96
Kakegawa, H., Nikawa, T., Tagami, K., Kamioka, H., Sumitani, K., Kawata, T., Drobni-Kosorok, M., Lenaric, B., Turk, V., and Katunuma, N. (1993) FEBS Lett. 321, 247–250
Khouri, H. E., Vernet, T., Menard, R., Parlati, F., Laflamme, F., Tessier, D. C., Gour-Salin, B., Thomas, D. Y., and Storer, A. C. (1991) Biochemistry 30, 8929–8936
Kirschke, H., and Wiederanders, B. (1994) Methods Enzymol. 244, 500–511
Kirschke, H., Brömmel, D., and Wiederanders, B. (1993) in Proteolysis and Protein Turnover (Bond, J. S., and Barrett, A. J., eds) pp. 33–37. Portland Press, London and Chapel Hill
Krane, S. M., and Simon, L. (1994) in Scientific American Medicine (Rubenstein, E., and Federman, D. D., eds) Vol. 3, pp. 1–26. Scientific American, Inc., New York
Leatherbarrow, R. J. (1987) Endriff, Elsevier Biosoft, Cambridge, United Kingdom
Madewicz, R. A., and Etherington, D. J. (1998) Biochem. J. 326, 433–440
Madewicz, R. A., Etherington, D. J., Kos, J., and V Turk, V. (1987) Colleague Ref. Res. 7, 295–304
Menard, R., Khouri, H. E., Plouffe, C., Laflamme, P., Dupras, R., Vernet, T., Tessier, D. C., Thomas, D. Y., and Storer, A. C. (1991) Biochemistry 30, 5531–5538
Page, A. E., Warburton, M. J., Chambers, T. J., Pringle, J. A. S., and Hayman, A. R. (1993) Biochim. Biophys. Acta 1126, 57–66
Rowan, A. D., Mason, P., Mach, L., and Morton, J. S. (1992) J. Biol. Chem. 267, 15993–15999
Reddy, M. V., Zhang, Q.-Y., and Weiss, S. J. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 3849–3853
Saneshige, T., Mano, H., Tezuka, K., Kakudo, S., Mori, Y., Honda, Y., Itabashi, A., Yamada, T., Miyata, K., Hakeda, Y., Ishii, J., and Kumeaga, M. (1995) Biochem. J. 309, 723–724
Shi, G.-P., Chapman, H. A., Bhai, S. M., DelLeeuw, C., Reddy, V. Y., and Weiss, S. J. (1995) FEBS Lett. 357, 129–134
Silver, I. A., Murrills, R. J., and Etherington, D. J. (1988) Exp. Cell. Res. 175, 266–276
Tezuka, K., Tezuka, Y., Maejima, A., Sato, T., Nenoto, K., Kamioka, H., Hakeda, Y., and Kumeaga, M. (1994) J. Biol. Chem. 269, 1106–1109
Tezuka, B., Dolenc, I., Turk, V., and Bieth, J. G. (1993) Biochemistry 32, 375–380
Vernet, T., Tessier, D. C., Richardson, C., Laliberte, F., Khouri, H. E., Bell, A. W., Storer, A. C., and Thomas, D. Y. (1990) J. Biol. Chem. 265, 16661–16666
Xin, X.Q., Gunesekera, B., and Mason, R. W. (1992) Arch. Biochem. Biophys. 299, 334–339
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