Release of Intact and Fragmented Osteocalcin Molecules from Bone Matrix during Bone Resorption in Vitro*

Received for publication, December 31, 2003, and in revised form, February 12, 2004
Published, JBC Papers in Press, February 16, 2004, DOI 10.1074/jbc.M314324200

Kaisa K. Ivaska‡§, Teuvo A. Hentunen¶, Jukka Vääriäniemi‡, Hannele Ylipahkala‡,
Kim Pettersson‡, and H. Kalervo Väänänen‡
From the Institute of Biomedicine, Department of Anatomy, and the Department of Biotechnology, University of Turku,
FIN-20520 Turku, Finland

© 2004 by The American Society for Biochemistry and Molecular Biology, Inc.
This paper is available online at http://www.jbc.org

Osteocalcin detected from serum samples is considered a specific marker of osteoblast activity and bone formation rate. However, osteocalcin embedded in bone matrix must also be released during bone resorption. To understand the contribution of each type of bone cell in circulating osteocalcin levels, we used immunoassays detecting different molecular forms of osteocalcin to monitor bone resorption in vitro. Osteoclasts were obtained from rat long bones and cultured on bovine bone slices using osteocalcin-depleted fetal bovine serum. In addition, human osteoclasts differentiated from peripheral blood mononuclear cells were used. Both rat and human osteoclasts released osteocalcin from bovine bone into medium. The amount of osteocalcin increased in the presence of parathyroid hormone, a stimulator of resorption, and decreased in the presence of bafilomycin A1, an inhibitor of resorption. The amount of osteocalcin in the medium correlated with a well-characterized marker of bone resorption, the C-terminal telopeptide of type I collagen (r > 0.9, p < 0.0001). The heterogeneity of released osteocalcin was determined using reverse phase high performance liquid chromatography, and several molecular forms of osteocalcin, including intact molecule, were identified in the culture medium. In conclusion, osteocalcin is released from the bone matrix during bone resorption as intact molecules and fragments. In addition to the conventional use as a marker of bone formation, osteocalcin can be used as a marker of bone resorption in vitro. Furthermore, bone matrix-derived osteocalcin may contribute to circulating osteocalcin levels, suggesting that serum osteocalcin should be considered as a marker of bone turnover rather than bone formation.

Osteocalcin (OC) is a 6-kDa noncollagenous protein produced by osteoblasts (1), osteocytes (2), and odontoblasts (3). Osteocalcin messenger RNA has also been detected in tissues other than bone, but it appears to be processed properly only in the bone microenvironment (4, 5). The structure of osteocalcin is characterized by three glutamic acid residues, which undergo vitamin K-dependent carboxylation. The vitamin K-dependent carboxylation of Glu residues provides osteocalcin with the ability to bind bone hydroxyapatite with a high affinity (6, 7). Osteocalcin is the second most abundant protein in the bone matrix, and it is highly conserved among all vertebrate species (8). The biological function of osteocalcin is probably related to the regulation of bone turnover and/or mineralization (9, 10).

The expression of osteocalcin is a marker of late osteoblast differentiation and is induced only after the expression of other osteoblastic markers such as alkaline phosphatase and type I collagen (11, 12). Newly synthesized osteocalcin is mostly (60–90%) adsorbed to the bone hydroxyapatite via the Gla residues, but a part of it leaks into the circulation where it can be detected (13, 14). Although osteoblasts synthesize only intact osteocalcin (15), osteocalcin may further undergo intracellular processing or be degraded after secretion, leading to the generation of smaller fragments. Only intact molecules are able to bind to the bone hydroxyapatite, and osteocalcin fragments lose their binding ability probably because of an altered conformation and subsequent loss of affinity for bone mineral (6, 16). Circulating osteocalcin has been widely used in clinical investigations as a marker of bone formation (17), whereas protein expression has served as an index of osteoblastic phenotype and bone formation in vitro (11).

Earlier studies have suggested that circulating osteocalcin originates exclusively from biosynthesis in osteoblasts and not from the breakdown of bone matrix (14, 18–20). However, later studies on patients with different metabolic bone diseases have suggested that not all of osteocalcin fragments are derived from the metabolism of osteocalcin in the circulation or peripheral organs but also from osteocalcin embedded in bone (21–23). Thus, part of osteocalcin found in the blood may also originate from the resorption process, when osteocalcin embedded in the bone matrix is released during bone degradation (Fig. 1). During bone resorption, osteoclasts secrete protons into the space between the bone surface and cells using vacuolar type H+ ATPase, and the acidification results in the dissolution of inorganic mineral. Organic bone matrix degradation is mediated by proteolytic enzymes, primarily cathepsin K, and the released material is endocytosed for further degradation in transcytotic vesicles in the resorbing osteoclast. Eventually, the degraded material is excreted into the extracellular space via a functional secretory domain (24, 25). Because osteocalcin

MALDI-MS, matrix-assisted laser desorption ionization time of flight mass spectrometry; TRITC, tetramethylrhodamine isothiocyanate.

This paper is available online at http://www.jbc.org

18361
Osteocalcin Released during Bone Resorption

is rather susceptible to proteolysis in vitro (16, 26), acids and proteases may also attack osteocalcin during bone degradation. Although the secretion of osteocalcin from osteoblasts has been widely studied, the release of osteocalcin molecules from the bone matrix and their potential contribution to the circulating osteocalcin pool has been discussed (27–29) but not clearly documented. If such a contribution exists, the circulating osteocalcin should rather be considered an indicator of bone turnover and not merely a marker of bone formation.

The purpose of the present study was to investigate whether osteocalcin detectable by immunoassays is released from bone during osteoclastic bone resorption, in addition to osteocalcin synthesized during bone formation. Furthermore, our aim was to evaluate different immunoassays for their capability of detecting bone matrix-derived osteocalcin in osteoclast cultures and to gain insight into the molecular forms of osteocalcin released during bone resorption in vitro.

EXPERIMENTAL PROCEDURES

Reagents—A Modified minimum essential medium, fetal bovine serum (FBS), 1 x HEPES solution, and antibiotics (penicillin and streptomycin) were purchased from Invitrogen. Macrophage colony-stimulating factor was purchased from R & D Systems, and the receptor activator of nuclear factor κB ligand (RANKL) and tumor necrosis factor α were from Peprotech. Dexamethasone, parathyroid hormone (PTH), bafilomycin A1 (BafA1, an inhibitor for vacuolar type H⁺ ATPase), ε-epoxysecurin-L-leucylamido-(4-guanidinobutane) (E64, an inhibitor for cysteine proteases), Hoechst 33258, and leukocyte activator of nuclear factor κB were purchased from R & D Systems, and the receptor activator of nuclear factor κB (NF-κB) were purchased from Invitrogen. Macrophage colony-stimulating factor, 20 ng/ml of RANKL, 10 ng/ml of tumor necrosis factor α, and 10⁻⁶ M dexamethasone for 12 days. Half of the medium was replaced with fresh medium containing 2-fold concentrations of cytokines every 4 days. Additionally, to study the release of the inorganic matrix in the absence of osteoclasts, some bovine bone slices were exposed to 0.6 M HCl at +4 °C for 24 h.

Osteocalcin-depleted Fetal Bovine Serum—The FBS used in the rat osteoclast cultures was depleted of bovine osteocalcin prior to use. Equal amounts of MAbs 8H12, 2H9, and 3H8 (1 mg of MAb mixture/1 ml of matrix) were coupled to a gel matrix (Affi-Gel 10; Bio-Rad) according to the manufacturer’s instructions using sterile reagents. FBS (14 ml) was mixed with the coupled matrix (1 ml) in an end-over-end rotator for 1 h at +4 °C and centrifuged for 10 min at 1,000×g. The supernatant, i.e. osteocalcin-depleted FBS, was collected and stored at −20 °C. The matrix was washed twice with PBS and osteocalcineluted with 0.5 M glycine-HCl, pH 2.5, in an end-over-end rotator for 15 min at +4 °C. The matrix was then washed twice with PBS prior to the preparation of the next batch.

Evaluation of Osteoclast Cultures—The osteocalcin immunoassays I-OC, M-OC, and T-OC described above and a competitive osteocalcin enzyme-linked immunosorbent assay (Rat-MID osteocalcin assay; Nor- dic Biosciences) were used to measure osteocalcin concentration in the medium. The amount of degraded bone matrix was assayed by measuring the C-terminal cross-linked telopeptide of type I collagen (CTX) using the Rat CTX-I kit (CrossLaps; Nordic) according to the manufacturer’s instructions. The activity of tartrate-resistant acid phosphatase isozyme 5b (TRACP5b) in the medium was assessed as described previously (35). The osteoclasts were fixed with 3% paraformaldehyde and cells were stained for TRACP enzyme activity with a leukocyte acid phosphatase kit. The nuclei were visualized with Hoechst staining, and the TRACP-positive multinucleated cells (at least 3 nuclei) were counted. In addition to the medium, the osteocalcin and calcium levels were also determined from the supernatants collected from the HCl-treated bone slices.

Immunostaining—The organic bone matrix components were visualized with fluorescein-labeled bone. The bone slices were incubated in a solution (pH 7.4) containing a 1:1000 dilution of fluorescein for 2 h with gentle stirring and then washed with PBS before use. Rat osteoclasts were cultured on labeled bone slices for 48 h, fixed with 3% paraformaldehyde, permeabilized with 0.1% Triton X-100 for 5 min on ice, and washed once with 2% bovine serum albumin

**Fig. 1. Possible sources of osteocalcin in circulation.** The newly synthesized osteocalcin (biosynthetic OC) is partly incorporated into the matrix and partly released into the circulation. Osteocalcin embedded in bone matrix is released during bone resorption (resorptive OC), but it is not precisely known how extensively osteocalcin is degraded during the resorption process. Osteocytes express osteocalcin, but their contribution to the circulating levels of osteocalcin is unknown.
Osteocalcin Released during Bone Resorption

Fig. 2. Osteocalcin is released into a culture medium during osteoclastic bone resorption in vitro. A, osteocalcin (T-OC) was detected in the culture medium of rat osteoclasts cultured on bovine bone slices. Significances compared with untreated cultures are shown as follows: a, p < 0.001; b, p < 0.01 (nonparametric Wilcoxon's test; significances below the plot are for the bone control and significances above the plot for the cell control). The data are combined from seven individual cultures, and the total number of replicates is 20–36 depending on the time point. B, treatment with PTH increased and treatment with BafA1 decreased the release of osteocalcin into medium. Significances compared with untreated cultures are shown as follows: a, p < 0.001; b, p < 0.05 (nonparametric Wilcoxon's test). The data are combined from six individual cultures, and the total number of replicates is 16–30 depending on the time point.

in PBS (BSA-PBS). The anti-osteocalcin MAb 3H8 (1000 ng/bone slice) diluted in 0.5% BSA-PBS was added and incubated for 45 min at room temperature. After washing with BSA-PBS, the cells were incubated with the Alexa 647-conjugated anti-mouse antibody and TRITC-phalloidin in BSA-PBS for 45 min at room temperature. After washing, the cells were evaluated with a Leica TCS-SP confocal laser scanning microscope equipped with an Argon-Krypton laser (Leica Microsystems). Fluorescein-labeled bone (A and C) and osteocalcin (B and D) can be found partially in the same compartments inside resorbing cells. Rat osteoclasts were stained for osteocalcin after a 2-day culture on fluorescein-labeled bovine bone slices and z sections (A and B) and x-y sections from the nuclear level (C and D) were obtained with a confocal laser scanning microscope. Actin staining was used to visualize cell boundaries (indicated with a dotted line) and to identify osteoclasts characterized by actin rings.

Fractionation of Osteocalcin from Cell Culture Media and Bovine Serum—The culture medium (4 ml) collected from the rat osteoclasts cultured for 5 days with 10 nM PTH and the supernatant obtained from the bone slices incubated with HCl at +4 °C for 24 h were used for the analysis. Further, osteocalcin was isolated from fetal bovine serum using the Affi-Gel 10 matrix coupled to the MAb8 H12, 2H9, and 3H8 as described above. The samples (the culture medium or the supernatant as such and the osteocalcin isolated from the serum) were extracted in solid phase extraction cartridges (Sep-Pak Plus C18, Milli-

Fig. 3. Osteocalcin was detected in a medium with immunoassays for various molecular forms of osteocalcin. The release of osteocalcin and the effect of BafA1 was detected by all three assays I-OC, M-OC, and T-OC. Significances of the bone control, the cell control, and the BafA1-treated culture (columns with patterns) compared with untreated osteoclasts cultured on bone (white columns) are shown as follows: a, p < 0.001; b, p < 0.01; c, p < 0.05 (nonparametric Wilcoxon's test with Bonferroni adjustment; n = 9 for bone only, n = 6 for cells only, n = 17 for osteoclasts on bone, and n = 16 for BafA1).

Statistical Analysis—Comparison between groups was performed with the nonparametric Wilcoxon's test using the Statistical Analysis System Enterprise Guide 2 program (SAS Institute). Bonferroni adjustment was used in multiple comparisons, and a p value of less than 0.05 was considered statistically significant. Because of normal distribution, one-way analysis of variance was used in the comparison of cultures performed with normal versus osteocalcin-depleted PBS, and Pearson correlation coefficients were used in correlation studies. All of the results are presented as the means ± S.E.
Osteocalcin Released during Bone Resorption

RESULTS

Osteocalcin (T-OC assay) was detected in the culture medium of rat osteoclasts after 2–3 days of culture on bovine bone, and the amount of osteocalcin increased in a time-dependent manner (Fig. 2A). The concentration of osteocalcin was higher in the osteoclast cultures than in the corresponding controls, and the difference was statistically significant after 3 days of culture (p = 0.0002 for the bone only control and p = 0.0007 for the cells only control, n = 36) and even more pronounced at the end of the culture period (p < 0.0001 for the bone only and p = 0.0014 for the cells only, n = 30). The release of osteocalcin into the medium was significantly increased when PTH, a known stimulator of bone resorption, was added to the medium, and osteocalcin was almost undetectable when BafA1, a potent inhibitor of the vacuolar type H+ ATPase and bone resorption, was included in the culture medium (Fig. 2A). Osteocalcin levels in the BafA1-treated cultures were similar to those of the controls at each time point (p > 0.05). In addition to the T-OC assay, osteocalcin was also detected with the I-OC and M-OC assays, and the levels of all detectable forms of the protein were significantly reduced in the presence of BafA1 (I-OC, p = 0.012; M-OC, p < 0.0001; and T-OC, p < 0.0001; n = 17, Fig. 3). Furthermore, an increase in osteocalcin in response to PTH was significant for all of the three assays (p values less than 0.0001; data not shown). Stimulation with PTH also resulted in the detection of osteocalcin when a competitive osteocalcin enzyme-linked immunosorbent assay was used (21.2 ± 2 ng/ml, n = 5). However, in the unstimulated cultures, the osteocalcin levels detected with this assay (22.8 ± 9.1 ng/ml, n = 5) did not differ from the reported detection limit (21.1 ng/ml). Osteocalcin was detected inside the resorbing osteoclasts by staining with a monoclonal antibody against a midmolecular epitope (MAb 3H8, epitope in the fragment 20–43) (Fig. 4, B and D). The bone matrix endocytosed from fluorescein-labeled matrix was also clearly detectable inside the cells (Fig. 4, A and C) and vesicles containing labeled bone partially co-localized with the vesicles positive for osteocalcin.

The osteocalcin detected in the culture medium of rat osteoclasts had a statistically significant positive correlation to the bone resorption rate as measured by CTX. The correlation coefficient for T-OC and CTX was 0.949 (p < 0.0001, n = 11) at the end of the culture period (day 5) in the cultures performed with osteocalcin-depleted FBS and not treated with stimulators or inhibitors (Fig. 5A). Osteocalcin detected in human osteoclast cultures also had a statistically positive correlation to bone resorption as evaluated by the CTX assay (Fig. 5B). The correlation coefficient at the end of the culture (day 12) was highest for T-OC (r = 0.934, p < 0.0001, n = 48) but also highly significant for the other osteocalcin assays (I-OC, r = 0.916, p < 0.0001; M-OC, r = 0.923, p < 0.0001) and osteocalcin enzyme-linked immunosorbent assay (p = 0.902, p < 0.0001).

The treatment of osteoclast cultures with two inhibitors of bone resorption, BafA1 and E64, resulted in distinct responses in putative bone degradation markers (Fig. 6). The amount of all detectable forms of osteocalcin was significantly reduced in the presence of BafA1 compared with the untreated cultures. In particular, the amount of M-OC and T-OC were decreased to 10% (p < 0.0001, n = 17) and also I-OC levels reduced to about 40% (p = 0.019, n = 17). In the presence of E64, the amount of osteocalcin was also reduced, but the inhibition was less pronounced. The M-OC and T-OC levels decreased to about

FIG. 5. Osteocalcin in medium correlates with bone degradation. Scatter plots of T-OC and CTX in a medium of rat osteoclasts cultured on bovine bone for 5 days (A) and human osteoclasts cultured on bovine bone for 12 days (B) are shown. The Pearson correlation coefficients were 0.949 (p < 0.0001, n = 11) and 0.934 (p < 0.0001, n = 48), respectively.

FIG. 6. The inhibitory effect of BafA1 and E64 is different on osteocalcin and similar on CTX. Significances for inhibited cultures (BafA1, gray columns; E64, white columns) compared with the untreated cultures (hatched columns) are shown above the columns as follows: a, p < 0.001; b, p < 0.01; c, p < 0.05; ns, p > 0.05 (not significant). The p values above the horizontal lines represent the significances between BafA1-treated cultures compared with the E64-treated cultures (nonparametric Wilcoxon’s test with Bonferroni adjustment). The data are pooled from three individual cultures; the average values above the horizontal lines represent the means (p values above the horizontal lines represents the means ± SD) of three individual cultures; the average values of untreated cultures (n = 17 for untreated, n = 16 for BafA1 and n = 12 for E64). The average values for untreated cultures were 1.4 ng/ml (I-OC), 4.5 ng/ml (M-OC), 8.2 ng/ml (T-OC), and 13.4 nm (CTX).
30–40% (p = 0.021 and 0.011, respectively), and a minor and nonsignificant decrease to about 75% was observed in I-OC levels. Thus, the levels of M-OC were significantly different after treatment with BafA1 and E64 (p < 0.0001) as were the levels of T-OC after similar treatments (p = 0.010). A similar, although not significant, trend was observed for I-OC. In con-

**Fig. 7.** Both intact osteocalcin and osteocalcin fragments were identified from osteoclast cultures and fetal bovine serum. Elution profiles from the fractionation of osteocalcin in rat osteoclast medium (10 mM PTH, 5 days) (A), released by demineralization with acid treatment (24 h, kinetics for release shown in inset) (B), or isolated from FBS with affinity chromatography (C). The fractions were measured with I-OC (solid squares), M-OC (open squares), and T-OC (triangles), and a representative example of an individual fractionation is displayed. All of the isolation and fractionation steps were performed twice from samples obtained from two independent cultures/purifications, and the elution profile was similar in shape in both independent runs. The numbers in C refer to the molecular forms of osteocalcin (residues 8–33 and 1–49) identified from the fractions indicated by arrows.
We studied the detachment of osteocalcin from the bone matrix during bone resorption in vitro. Osteocalcin was clearly released into the medium during osteoclastic resorption both in the rat and human in vitro models we used. The concentration of osteocalcin increased in the presence of PTH, a known stimulator of bone resorption, and was almost undetectable in the presence of bafilomycin A1, an inhibitor of bone resorption. A minor amount of osteocalcin was detected in medium incubated with bone slices alone, but the level was not increased after the first 24 h. Also, the rat bone cells cultured on glass coverslips did not produce detectable levels of biosynthetic osteocalcin. Osteocalcin was detected with assays measuring either intact osteocalcin exclusively (I-OC) or those also detecting fragments in addition to intact molecules (M-OC and T-OC), and these results were also reproducible by a commercially available enzyme-linked immunosorbent assay. The concentration of osteocalcin in the culture medium demonstrated a strong (\( r > 0.9 \)) and highly significant (\( p < 0.0001 \)) correlation to the concentration of CTX, which is frequently used to quantify resorption in bone culture supernatants. The correlation between osteocalcin and CTX was observed both in the rat and human osteoclast cultures. Taken together, these results suggest that the osteocalcin detected by the immunoassays is derived from degraded matrix and can thus be used as an index of bone resorption in vitro. The correlation to CTX was nearly similar with all osteocalcin assays, further suggesting that several different molecular forms of osteocalcin can be used in monitoring cultures. We have previously reported that the same immunoassays are suitable in monitoring osteocalcin production in rat primary osteoblasts (45), indicating that the same osteocalcin immunoassays can be applied to monitor both de novo synthesized osteocalcin in osteoblast cultures, as well as the bone matrix-derived osteocalcin in osteoclast cultures.

In addition to the immunoassays, osteocalcin was demonstrated inside bone-resorbing osteoclasts by immunostaining, and intracellular osteocalcin was predominantly located in the same vesicles as the endocytosed bone matrix. Although the labeling of bone with fluorescein has probably produced small amounts of labeled osteocalcin, which can be responsible for some of the co-localization, this does not invalidate the conclusion that osteocalcin can be detected inside osteoclasts in the very same compartments as the proteins originally present in the bone matrix. In addition to intracellular vesicles, osteocalcin immunostaining was also observed at the bottom of the resorption lacunae but not on intact bone surfaces, suggesting that mineral dissolution was obligatory to expose the binding epitopes. The immunoassay results and microscopic data together support a concept for the release of immunodetectable osteocalcin during bone resorption. In agreement with our results, Kurihara et al. (46) have provided evidence for osteocalcin immunoreactivity in the supernatant of human bone particle-derived osteoclasts cultured on human bone slices.

**DISCUSSION**

Since the discovery of osteocalcin in the late 1970s (13, 37, 38), it has been used as a specific marker of osteoblast activity both in vivo (17, 39, 40) and in vitro (1, 41) because of the restricted expression in the mature cells of osteoblastic lineage (15, 42, 43). Osteocalcin content in adult bone has been reported to range from 0.28 (human) to 2–2.5 (cow) mg/g of dry bone, and it represents up to 20% of noncollagenous bone matrix proteins (8, 44). During bone remodeling, osteocalcin embedded in the matrix is exposed to osteoclastic bone resorption and to the proteolytic microenvironment needed for bone degradation. In vitro, osteocalcin is susceptible to proteolysis and several proteases, e.g. plasmin, trypsin, and cathepsins can cleave human osteocalcin into smaller fragments (16, 26). However, because the molecular weight of osteocalcin is small (~6 Da) compared with many other bone matrix proteins, it may not require extensive cleavage for detachment from the matrix in vivo.

**Osteocalcin Released during Bone Resorption**

| HPLC elution time | N-terminal sequencing | Observed M+H+ | Bovine OC fragment | Theoretical M+H+ |
|------------------|-----------------------|--------------|--------------------|-----------------|
| 43 min           | Ala¹Asp¹⁴             | 2713         | 8–31               | 2713            |
| 45 min           | ND                    | 2896         | 8–33               | 2897            |
| 47 min           | Ala¹               | 3595         | 8–39               | 3595            |
| 49 min           | Ala¹               | 4834         | 8–49               | 4834            |
| 5720 Da          | ND                   | 1–49         | 5719               |

¹ Because γ-carboxylation is destroyed in MALDI-MS and not included in observed ions (51,52), all calculations of theoretical molecular masses were performed using the sequence of bovine osteocalcin (average molecular masses) with glutamic acid at positions 17, 21, and 24 instead of γ-carboxyglutamic acid.

² ND, not determined.
with a sandwich assay for the N-terminal epitope of human osteocalcin.

The correlation between CTX and intact osteocalcin indicated that part of the osteocalcin was able to escape complete degradation in osteoclasts and was released from resorption as intact, unfragmented molecules. This was further supported by the HPLC fractionation of osteocalcin from the osteoclast culture medium, because the largest peak in the elution profile was positive for the I-OC assay and thus consisted of intact osteocalcin molecules. Two smaller peaks in the elution profile could not be identified due to the small amount of protein, but on the basis of earlier elution time they most likely consist of osteocalcin fragments. Peaks were eluted approximately at the same time (45 and 47 min) as two fragments of serum osteocalcin. Thus, the peaks in the osteoclast culture medium might contain osteocalcin fragments similar to those detected in se-

**Fig. 8.** Bone resorption was not disturbed when FBS in the culture medium was replaced by osteocalcin-depleted FBS. TRACP-positive multinucleated osteoclasts were observed in cultures using both untreated FBS (A) and osteocalcin-depleted FBS (B). The original magnification is 10×. There was no difference in the number of osteoclasts/bone slice \( p = 0.67, n = 8 \) nor in the activity of TRACP5b \( p = 0.89, n = 4 \) or the amount of CTX \( p = 0.92, n = 11 \) released into the medium (C). The statistics have been calculated with one-way analysis of variance.
Osteocalcin Released during Bone Resorption

It is well documented that osteoblasts produce osteocalcin, which is used as a marker of bone formation. We have demonstrated that osteocalcin is released during bone resorption as well as being produced by osteoblasts during bone formation. A part of osteocalcin escapes the proteolytic degradation during bone resorption and is released both as intact molecules and fragments. The detection of osteocalcin in the culture medium provides a useful tool for monitoring the bone resorption rate in osteoclast cultures. These results also suggest that a fraction of intact osteocalcin and osteocalcin fragments in the circulation may actually be derived from bone resorption, although it is difficult to estimate the relative proportion of serum osteocalcin that would come from each process in a clinical setting. In conclusion, serum osteocalcin should preferentially be considered a bone turnover marker instead of a pure marker of bone formation.

Acknowledgment—Terhi J. Heino is acknowledged for reading the manuscript.

REFERENCES

1. Berosad, J. N., Gallagher, J. A., Poser, J. W., and Russell, R. G. (1984) Metab. Bone Dis. Relat. Res. 5, 229–234
2. Masson, D. J., Hillam, R. A., and Skerry, T. M. (1996) J. Bone Miner. Res. 11, 3560–3577
3. DiMuzio, M. T., Bhowm, M., and Butler, W. T. (1983) Biochem. J. 216, 249–257
4. Thiede, M. A., Smock, S. L., Petersen, D. N., Grasser, W. A., Thompson, D. D., and Nishimoto, S. K. (1994) Endocrinology 135, 929–937
5. Jung, C., Ou, Y. C., Yeung, F., Frierson, H. F., and Kao, C. (2001) Gene 271, 143–150
6. Dowd, T. L., Rosen, J. F., Li, L., and Gundberg, C. M. (2003) Biochemistry 42, 7769–7779
7. Hoang, Q. Q., Schieri, F., Howard, A. J., and Yang, D. S. (1994) Nature 365, 977–980
8. Hauschka, P. V., Lian, J. B., Cole, D. E., and Gundberg, C. M. (1989) Physiol. Rev. 69, 990–1047
9. Boskey, A. L., Gudaleta, S., Gundberg, C., Doty, S. B., Ducy, P., and Karsenty, G. (1998) Bone 23, 137–146
10. Ducy, P., Desbois, C., Boyce, P., Binero, G., Story, B., Dunstan, C., Smith, E., Bonadio, J., Goldstein, S., Gundberg, C., Bradley, A., and Karsenty, G. (1996) Nature 382, 448–452
11. Owen, T. A., Aronow, M., Shalhoub, V., Barone, L. M., Wilming, L., Tassiniari, M. S., Kennedy, M. B., Peakwine, S., Lian, J. B., and Stein, G. S. (1990) J. Cell. Physiol. 143, 420–430
12. Bellows, C. G., Reimers, S. M., and Heersche, J. N. (1999) Cell Tissue Res. 297, 249–259
13. Price, P. A., Poser, J. W., and Raman, N. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 3374–3378
14. Price, P. A., Williamson, M. K., and Lohtringer, J. W. (1981) J. Biol. Chem. 256, 12760–12766
15. Celente, A. J., Rosen, V., Buecker, J. L., Krix, R., Wang, E. A., and Wozney, J. M. (1986) EMBO J. 5, 1885–1890
16. Novak, J. F., Hayes, J. D., and Nishimoto, S. K. (1997) J. Bone Miner. Res. 12, 1035–1042
17. Power, M. J., and Fottrell, P. F. (1991) Crit. Rev. Clin. Lab. Sci. 28, 287–335
18. Brown, J. P., Delmas, P. D., Malaval, L., Edouard, C., Chapuy, M. C., and Meunier, P. J. (1984) Lancet 1, 1091–1093
19. Biggs, B. L., Tsai, R. S., and Mann, K. G. (1986) J. Bone Miner. Res. 1, 539–542
20. Charles, P., Poser, J. W., Mosekilde, L., and Jensen, F. T. (1985) J. Clin. Invest. 76, 2254–2258
21. Gundberg, C. M., and Weinstein, R. S. (1986) J. Clin. Invest. 77, 1762–1767
22. Taylor, A. K., Linkhart, S., Mohan, S., Christenson, R. A., Singer, F. R., and Baylink, D. J. (1990) J. Clin. Endocrinol. Metab. 70, 467–472
23. Gundberg, C. M., Groetz, P. D., Conlin, P. R., Chen, C. J., Brown, E. M., Johnson, P. J., and LeBoff, M. S. (1991) J. Clin. Endocrinol. Metab. 72, 438–443
24. Nielsen, S. A., and Horton, M. A. (1997) Science 276, 268–269
25. Sale, J., Lehenkari, P., Mulari, M., Metsikko, K., and Vaananen, H. K. (1997) Science 276, 270–273
26. Baumgauss, R., Williamson, M. K., and Price, P. A. (1997) J. Bone Miner. Res. 12, 447–455
27. Christenson, R. H. (1997) Clin. Biochem. 30, 573–593
28. Fehr, B., Dunstan, C. R., and Seibel, M. J. (2005) J. Clin. Endocrinol. Metab. 88, 5059–5075
29. Chen, J. T., Hosoda, K., Hasumi, K., Ogata, E., and Shiraki, M. (1994) J. Bone Miner. Res. 11, 1784–1792
30. Heersche, J., Kakouma, S. M., Matikainen, M. T., Karp, M., Lovgren, T., Vaananen, H. K., and Pettersson, K. (1996) J. Bone Miner. Res. 11, 1165–1175
31. Lakkakorpi, P., Tuukkanen, J., Hentunen, T., Jarvelin, K., and Vaananen, K. (1989) J. Bone Miner. Res. 4, 817–825
32. Boyd, A., Ali, N. N., and Jones, S. J. (1984) Br. Dent. J. 156, 216–220
33. Chambers, T. J., Revell, P. A., Fuller, K., and Athanason, N. A. (1984) J. Cell. Sci. 66, 383–399
34. Hentunen, T., and Vaananen, H. K. (2001) J. Bone Miner. Res. 16, S377
35. Aalato, S. L., Halleen, J. M., Hentunen, T. A., Monkkonen, J., and Vaananen,
36. Ivaska, K. K., Hellman, J., Likojarvi, J., Kakonen, S. M., Gerthem, P., Åkesson, K., Obrant, K. J., Pettersson, K., and Vaananen, H. K. (2003) Biochem. Biophys. Res. Commun. 306, 973–980
37. Price, P. A., Otsuka, A. A., Poser, J. W., Kristaponis, J., and Raman, N. (1976) Proc. Natl. Acad. Sci. U. S. A. 73, 1447–1451
38. Hauschka, P. V., and Gallop, P. M. (1977) in Calcium Binding Proteins and Calcium Function (Wasserman, R., ed) pp. 338–347, Elsevier/North-Holland, Amsterdam, The Netherlands
39. Price, P. A., and Nishimoto, S. K. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 2234–2238
40. Price, P. A., Parthemore, J. G., and Deftos, L. J. (1980) J. Clin. Invest. 66, 878–883
41. Lian, J. B., Coutts, M., and Canalis, E. (1985) J. Biol. Chem. 260, 8706–8710
42. Price, P. A., and Baukol, S. A. (1980) J. Biol. Chem. 255, 11660–11663
43. Lian, J., Stewart, C., Puchacz, E., Mackowiak, S., Shalhoub, V., Collart, D., Zambetti, G., and Stein, G. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 1143–1147
44. Gallop, P. M., Lian, J. B., and Hauschka, P. V. (1980) N. Engl. J. Med. 302, 1460–1466
45. Ivaska, K. K., Ylipahkala, H., Hentunen, T. A., and Vaananen, H. K. (2002) J. Bone Miner. Res. 17, S407
46. Kurihara, N., Hosoda, K., Tatsumi, J., Yamaji, T., Hoshihara, E., F., A., and Ikeda, K. (1998) J. Bone Miner. Metab. 16, 11–16
47. Farrugia, W., and Melick, R. A. (1986) Calcif. Tissue Int. 39, 234–238
48. Alberti, G., Pulignano, I., Proietta, M., Tritapepe, L., Cigognetti, L., Menichetti, A., Russo, A., de Michele, L. V., Corvisieri, P., and Minisola, S. (2000) Clin. Physiol. 20, 122–125
49. Gundberg, C. M., Clough, M., and Mort, J. S. (2002) J. Bone Miner. Res. 17, S406
50. Garnero, P., Girmaux, M., Seguin, P., and Delmas, P. D. (1994) J. Bone Miner. Res. 9, 255–264
51. Prorok, M., Warder, S. E., Blaxland, T., and Castellino, F. J. (1996) Biochemistry 35, 16528–16534
52. Kalume, D. E., Stenflo, J., Cserwicz, E., Hambe, B., Furie, B. C., Furie, B., and Roepstorff, P. (2000) J. Mass Spectrom. 35, 143–156
Release of Intact and Fragmented Osteocalcin Molecules from Bone Matrix during Bone Resorption in Vitro
Kaisa K. Ivaska, Teuvo A. Hentunen, Jukka Vääräniemi, Hannele Ylipahkala, Kim Pettersson and H. Kalervo Väänänen

J. Biol. Chem. 2004, 279:18361-18369.
doi: 10.1074/jbc.M314324200 originally published online February 16, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M314324200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 51 references, 12 of which can be accessed free at http://www.jbc.org/content/279/18/18361.full.html#ref-list-1