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Neutrophil collagenase activation: The role of oxidants and cathepsin G

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Abstract

The events leading to neutrophil collagenase activation *in vivo* were analyzed using phorbol myristate acetate (PMA) stimulated neutrophil supernatant. Under the conditions when this supernatant was incubated with the serine proteinase inhibitor, phenylmethylsulfonyl fluoride (PMSF), and then treated with the oxidant, hypochlorous acid (HOCl), collagenase was activated. When cathepsin G, a known activator of neutrophil collagenase, was also present, less HOCl was required to activate the latent collagenase. These experiments support the conclusion that activation of neutrophil collagenase occurs *in vivo* by both an oxidant and an enzymatic mechanism where the effectiveness of oxidants is enhanced by cathepsin G.

Introduction

Matrix damage during inflammation, in part, is caused by neutrophil collagenase, a latent metalloenzyme whose mechanism of activation *in vivo* is poorly understood. Fibroblast collagenase, a close relative of neutrophil collagenase, has been reported to be activated by the serine proteinase, plasmin [1]. The serine proteinase from neutrophils, cathepsin G, has been reported to activate neutrophil collagenase in PMA stimulated supernatants [2]. Cathepsin G cleaves a 78 amino acid residue N-terminal propeptide resulting in the activation of the proenzyme [3]. Oxidants, such as HOCl, have also been reported to activate procollagenase in neutrophil supernatants [4]. The rationale of the following experiments was designed to understand the means by which neutrophil collagenase is activated *in vivo*.

Materials and methods

Collagenase assay

Neutrophils (1×10^7 cells/ml), isolated according to the method of Goldstein et al. [5], were stimulated with PMA (60 ng/ml) purchased from Sigma (St. Louis, MO) to induce proteinase secretion. The cells were pelleted and the medium was treated with 5 m*M* PMSF in some cases, and incubated with either 0.5 *M* p-aminophenylmercuric acetate (APMA) or varying concentrations of HOCl and/or 5 μg/0.5 ml cathepsin G, purchased from Elastin Products Company (Owensville, Missouri). Buffer (0.05 *M* Tris, 0.01 *M* $CaCl_2$ and 0.15 *M* NaCl, pH 7.5) was added along with [^{14}C]-labeled collagen [6]. This mixture was then incubated for 20 h at 25 °C. The samples were lyophilized and analyzed by polyacrylamide gel electrophoresis in SDS.

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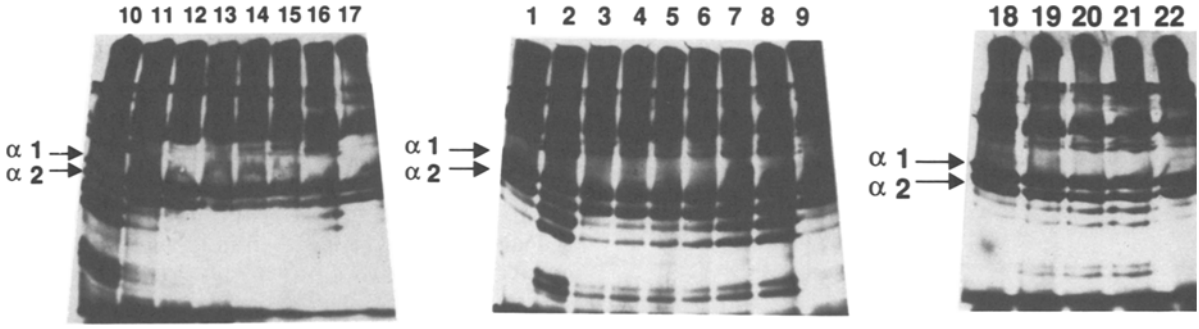

Figure 1
Collagenase activation in the presence of exogenous cathepsin G and HOCl. Untreated and PMSF-treated supernatants were incubated with HOCl in the absence and/or presence of cathepsin G for 30 minutes at 37 °C. Collagenase activity was measured by visualizing radiolabeled type I collagen degradation on 6% polyacrylamide slab gels in SDS. Final reaction volume was 0.5 ml. Lanes 1–8 contain untreated supernatant in addition to the substances listed in the legend. Lanes 9–21 contain PMSF-treated supernatant in addition to the substances listed in the legend. Panel a: Lane 1 PMA-stimulated supernatant alone, Lane 2 APMA (0.5 m*M*), Lane 3 cathepsin G (5 μg), Lane 4 HOCl (1 μ*M*), Lane 5 HOCl (10 μ*M*), Lane 6 HOCl (25 μ*M*), Lane 7 HOCl (50 μ*M*), Lane 8 HOCl (100 μ*M*), Lane 9 PMSF-treated supernatant alone. Panel B: Lane 10 APMA (0.5 m*M*), Lane 11 cathepsin G (5 μg), Lane 12 HOCl (1 μ*M*), Lane 13 HOCl (10 μ*M*), Lane 14 HOCl (25 μ*M*), Lane 15 HOCl (50 μ*M*), Lane 16 HOCl (100 μ*M*), Lane 17 HOCl (1 μ*M*) + cathepsin G (5 μg). Panel C: Lane 18 HOCl (10 μ*M*) + cathepsin G (5 μg), Lane 19 HOCl (25 μ*M*) + cathepsin G (5 μg), Lane 20 HOCl (50 μ*M*) + cathepsin G (5 μg), Lane 21 HOCl (100 μ*M*) + cathepsin G (5 μg), Lane 22 collagen substrate alone.

Results

In order to assess the role of oxidants in neutrophil collagenase activation, procollagenase activation was analyzed using HOCl under the conditions where cathepsin G was inactive. Neutrophils were treated with PMA to induce proteinase secretion. The supernatant was then incubated with PMSF to inhibit endogenous serine proteinase activity. Excess PMSF was removed by dialysis and activation with HOCl was performed. Supernatant that was not treated with PMSF was examined as a comparison. Collagenase was secreted with very little activity (lanes 1 & 9, Fig. 1). The activity observed in these lanes was evaluated as 0% activity. The procollagenase was activated by the organomercurial, APMA, in both the untreated supernatant (lane 2, Fig. 1) and the PMSF-treated supernatent (lane 10, Fig. 1). Cathepsin G was also able to activate procollagenase (lanes 3 & 11, Fig. 1). Hypochlorous acid was able to activate the procollagenase in supernatants not treated with PMSF in a dose-dependent manner, 1 μ*M* to 100 μ*M* (lanes 4–8, Fig. 1). Supernatants treated with PMSF exhibited collagenase activity as a result of HOCl only at the extreme concentration of 100 μ*M* (lane 16, Fig. 1). When cathepsin G was added to the PMSF-treated supernatants activation was observed at a concentration of 25 μ*M* HOCl (lane 19, Fig. 1). This experiment supports the hypothesis that cathepsin G is likely to be responsible for neutrophil collagenase activation *in vivo* and the effectiveness of oxidants is enhanced by cathepsin G.

Discussion

During the inflammatory response, neutrophils infiltrate an affected site, expel the contents of their secretory granules and release oxygen derived free radicals. The question to be addressed is how the neutrophil utilizes the contents of its secretory granules, in addition to the oxidants which it is capable of producing, to execute the activation of neutrophil collagenase.

During this study, the oxidizing agent, HOCl, was used to analyze the efficiency of oxidants in activating neutrophil collagenase. This particular oxidant was chosen since neutrophils produce up to 20 $\mu M/10^7$ cells of HOCl [7]. Neutrophil collagenase was shown to be activated by HOCl, in PMA stimulated supernatants, as was shown previously [4]. When the PMA stimulated neutrophil supernatants were treated with the serine proteinase inhibitor, PMSF, activation of neutrophil collagenase by HOCl was reduced. Cathepsin G was then added to the PMSF-treated supernatants and acti-

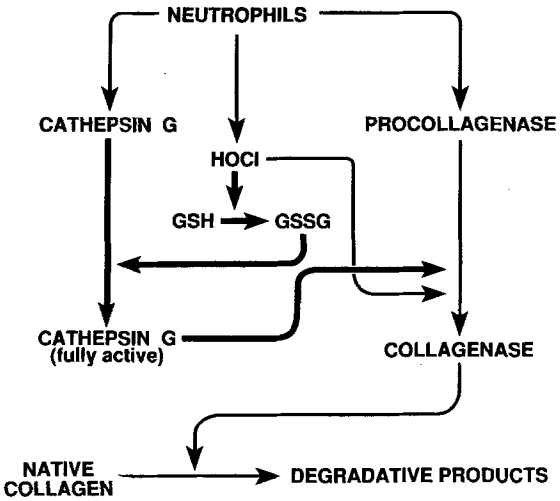

Figure 2
Proposed mechanism for procollagenase activation *in vivo*. Neutrophils secrete cathepsin G, procollagenase, and oxidants, including HOCl. 1. The oxidant, HOCl, can activate procollagenase directly by oxidizing the critical cysteine exposing the active site of the enzyme. 2. Cathepsin G, stabilized in an active form GSSG in the oxidizing environment of the neutrophil, cleaves and activates the procollagenase.

vation by HOCl was more efficient than observed in PMSF treated supernatant without the addition of exogenous cathepsin G.

Since cleavage of native collagen is the rate-limiting step in collagen degradation, it is logical that collagenase can be activated by more than one pathway. Figure 2 demonstrates that oxidants, such as HOCl, can activate procollagenase directly by oxidizing the critical cysteine exposing the active site of the enzyme. In addition to a direct effect by oxidants, this study implicates a proteinase cascade in the activation of neutrophil collagenase. Since it is known that cathepsin G is stabilized in an active form by oxidized glutathione [8], the oxidizing environment of the neutrophil may provide a dual pathway where cathepsin G activates procollagenase through a proteolytic cleavage.

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