Preferential binding of collagenase to α_2-macroglobulin in the presence of the tissue inhibitor of metalloproteinases

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The binding of collagenase to both α_2-macroglobulin and the tissue inhibitor of metalloproteinases was studied using purified materials. Collagenase bound preferentially to α_2-macroglobulin although no transfer of collagenase to α_2-macroglobulin occurred if the enzyme was first mixed with the tissue inhibitor of metalloproteinases. The sequences of amino acids in both inhibitors likely to be responsible for the binding of collagenase are discussed and compared to the cleavage site in the collagen molecule.

Collagenase *α_2-Macroglobulin* *Cleavage site* *Connective tissue breakdown* *Enzyme-inhibitor complex* *TIMP*

1. INTRODUCTION

The enzyme collagenase is a metalloproteinase which specifically cleaves the triple helical collagen molecule at one point to give characteristic 1/4 and 3/4 fragments. This enzyme is thought to be involved in normal tissue remodelling, and consequently a number of control mechanisms exist to limit its extracellular activity.

Various workers have investigated the naturally occurring metalloproteinase inhibitors. Using either cell or explant culture techniques they detected an inhibitor of metalloproteinases released from the tissues or cells in the early days of culture [1]. This inhibitor, named the tissue inhibitor of metalloproteinases (TIMP), was subsequently purified to homogeneity from rabbit bone culture medium [2], human tendon culture medium [3] and human skin fibroblast culture medium [4]. All these inhibitors were glycoproteins with an M_r of approx. 27500 and they specifically inhibited metalloproteinases. TIMP interacted with the active form of collagenase to form a tight binding enzyme-inhibitor complex which had an apparent M_r of 54000. It appeared that this interaction was irreversible and the enzyme once bound by inhibitor could not further degrade collagen [5]. In addition, the inhibitor formed a similar tight-binding complex with the proteoglycanase and gelatinase purified from rabbit bones [6].

Human body fluids contain low levels of TIMP [7,8] and we have recently detected and purified this inhibitor from rheumatoid synovial fluid [9,10]. However, in both serum and synovial fluid the majority of the collagenase inhibitor activity (>95%) towards collagenase is due to the presence of α_2-macroglobulin (α_2M) [11], a proteinase inhibitor which can inhibit all the four classes of proteinases [12]. As both α_2M and TIMP are present in rheumatoid synovial fluid [9] we decided to investigate the binding of collagenase to both inhibitors.

2. MATERIALS AND METHODS

Ultrogel AcA 44 was obtained from LKB instruments, Croydon, England. All chemicals and reagents were of the highest analytical grade available and were obtained from the suppliers previously described [2,5]. Collagenase was iodinated using the Bolton and Hunter reagent as described [13]. TIMP was prepared from rabbit bone culture medium [2]. Collagenase was

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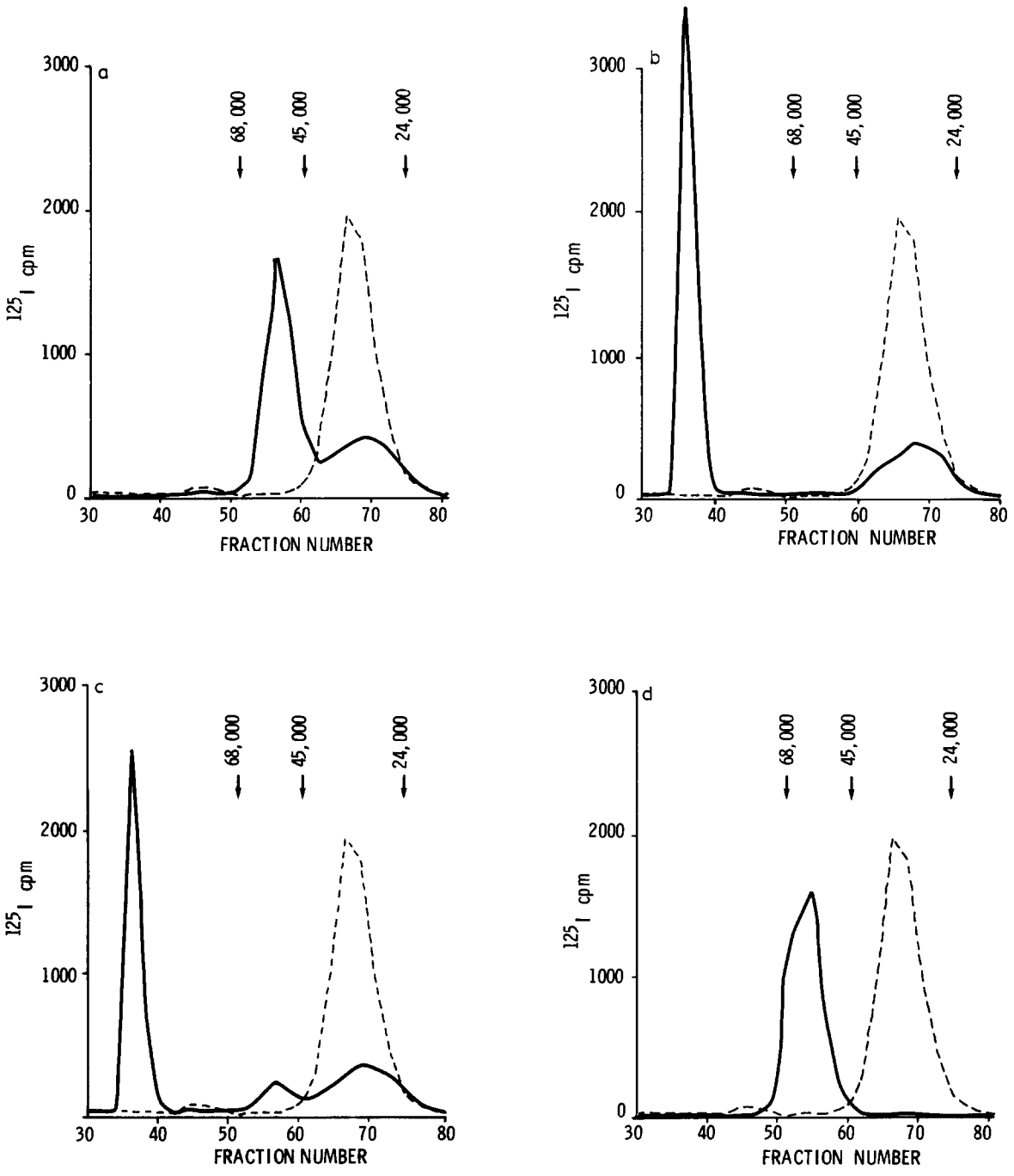

Fig.1. Gel filtration of ^{125}I-labelled collagenase with and without inhibitors. A sample of ^{125}I-labelled collagenase was divided into five equal portions. One was loaded onto a column of Ultrogel AcA 44 (1.6 × 88 cm) and eluted at a flow rate of 12 ml/h. Fractions (1.8 ml) were collected and counted for ^{125}I radioactivity. The other four portions were mixed with equimolar amounts of (a) TIMP, (b) α_2M, (c) TIMP + α_2M, (d) TIMP followed by α_2M. The elution profiles are shown for collagenase chromatographed with (——) and without (– – –) inhibitors.

prepared from pig synovial tissue culture medium [13] and the interaction experiments of enzyme and inhibitor as described by Cawston et al. [5]. Collagenase activity was measured following the digestion of ^{14}C type I collagen [14]. For collagenase inhibitor assays a known amount of collagenase was added and assayed in the presence of inhibitor samples. A unit of collagenase degrades 1 μg of collagen/min at 37°C and 1 unit of inhibitor inhibits 2 units of collagenase by 50% [5]. α_2M was prepared by the method of Barrett [15].

3. RESULTS

Collagenase is known to form a tight-binding complex (M_r 54000) with TIMP that is stable to gel filtration [5]. Collagenase was labelled with ^{125}I and applied to an Ultrogel AcA 44 gel filtration column. The ^{125}I-labelled peak of collagenase eluting at M_r 30000 was pooled. Aliquots of this pool were then reapplied to the column both alone and with sufficient TIMP to just inhibit all the enzyme present. In the presence of TIMP the labelled collagenase eluted at an M_r of 54000 (fig.1a). When an equimolar amount of sufficient α_2M was added to the collagenase to just inhibit all the enzyme a different elution profile was obtained (fig.1b). All of the ^{125}I-labelled collagenase eluted at the void volume of the column indicating that it was bound to the large α_2M molecule. Fig.1c illustrates the results obtained when sufficient TIMP and α_2M each to inhibit completely the collagenase were mixed together and then the ^{125}I-labelled collagenase was added to this mixture and subsequently gel filtered. The collagenase was preferentially bound to the α_2M and no peak of radioactivity was found at M_r 54000. A subsequent experiment (fig.1d) mixed an equimolar portion of TIMP and ^{125}I-labelled collagenase. After incubation at room temperature an equimolar portion of α_2M was added and the mixture gel filtered (fig.1d). The profile indicates that after binding to TIMP the collagenase could not be transferred to α_2M as all of the ^{125}I-labelled protein eluted at M_r 54000.

4. DISCUSSION

Previous studies on the interaction of TIMP with collagenase have indicated that the collagenase binds tightly and specifically to the inhibitor [5]. Although Stricklin and Welgus [4] originally suggested that collagenase only bound TIMP in the presence of collagen they have recently reported [16] that a complex, stable to gel filtration, is rapidly formed. Other workers have also reported that collagenase rapidly binds to TIMP [3]. As TIMP appears to be produced by connective tissue cells to control specifically the local extracellular activity of the metalloproteinases and α_2M is a proteinase inhibitor in general confined to the bloodstream and with a broad specificity for all four classes of proteinases we were surprised to find that collagenase bound to α_2M when in competition with TIMP. The sequence of amino acids reported to be responsible for the binding of collagenase by α_2M was suggested to be the Gly-Pro-Glu-Gly-Leu sequence found close to the cleavage site of α_2M [17]. This resembled the Gly-Pro-Glu-Gly-Ile sequence at the cleavage site of the collagen α-chain. In TIMP no similar sequence appears to be present except for an Arg-Glu-Pro-Gly-Leu sequence near the C-terminal end [18]. Whether or not this is the cleavage site or binding site for collagenase is not known but it is interesting to note that the corresponding sequence in TIMP is less homologous to the collagen cleavage site than that in α_2M and this may be why collagenase binds preferentially to α_2M.

These results differ from those reported [19] where human polymorphonuclear leukocyte collagenase was incubated for 24 h with a mixture of human plasma B_1-anticollagenase and an excess of human α_2M. Only 10% of the collagenase bound to the α_2M whilst the remainder was firmly bound to the B_1-anticollagenase. However, it is not clear if the inhibitor described by Macartney and Tschesche [19] as B_1-anticollagenase is the same protein as TIMP [20].

It is apparent too from these results that the binding of collagenase to α_2M is rapid and it obviously occurs more quickly than the binding to TIMP which is known to be fast [5]. Werb et al. [21] reported that rather slow binding to collagenase occurred although Borth [22] reported a rapid binding and cleavage of the α_2M to the fast form by collagenase.

These results do not necessarily suggest that α_2M has a major role in the inhibition of collagenase. It is likely that TIMP's main role is in a very local

area immediately around the cell from which, in connective tissue, α_2M is excluded because of its size. However, the results do suggest that α_2M can rapidly inactivate collagenase in acute inflammatory situations and so limit tissue damage. In order to determine the extent to which either inhibitor is responsible for prevention of collagenolytic activity it would be interesting to analyse biological fluids for the amounts of either α_2M or TIMP bound to collagenase.

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