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Effect of DNase on the activity of neutrophil elastase, cathepsin G and proteinase 3 in the presence of DNA

Jérôme Duranton[a], Didier Belorgey[a], Jacqueline Carrère[b], Lionel Donato[c], Thomas Moritz[a], Joseph G. Bieth[a,*]

[a] *Laboratoire d'Enzymologie, INSERM Unité 392, Faculté de Pharmacie, Université Louis Pasteur de Strasbourg, F-67400 Illkirch, France*
[b] *Hôpital Renée Sabran, 83406 Giens, France*
[c] *Service de Pédiatrie II, Hôpital Universitaire de Hautepierre, 67200 Strasbourg, France*

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Abstract It has been shown previously that DNA binds and inhibits neutrophil elastase (NE). Here we demonstrate that DNA has a better affinity for neutrophil cathepsin G (cat G) than for NE and is a better inhibitor of cat G than of NE. DNase-generated <0.5 kb DNA fragments inhibit NE and cat G as potently as full length DNA. This rationalises our observation that administration of DNase to cystic fibrosis patients does not enhance the NE and cat G activity of their lung secretions. Neutrophil proteinase 3 is not inhibited by DNA and might thus be the most harmful proteinase in inflammatory lung diseases.

Key words: Elastase; Cathepsin G; Proteinase 3; DNA; DNase; Cystic fibrosis

1. Introduction

The azurophil granules of neutrophils store neutrophil elastase (NE), neutrophil cathepsin G (cat G) and neutrophil proteinase 3 (PR3), three serine proteinases which are able to cleave lung extracellular matrix proteins such as elastin, collagen, fibronectin and laminin and to cause extensive lung tissue damage in the animal [1]. NE is thought to play a major role in the pathogenesis of the pulmonary manifestation of cystic fibrosis [2]. In this disease excessive phagocytosis and cell death of neutrophils lead to a permanent release of granule proteinases and nuclear DNA [2]. The latter binds and partially inhibits NE in vitro [3,4] and may therefore partially restore the antielastase defence of airways secretions. It is not known whether DNA also inhibits cat G and PR3.

Cystic fibrosis is characterised by an accumulation of viscous secretions which contribute to the bacterial colonisation of airways. Since neutrophil-derived DNA is a major contributor to this viscosity, patients are now treated with aerosols of DNase which improve their lung function [5]. This treatment depolymerises the airways DNA down to an average size of about 0.4 kb [6]. A number of investigators have measured the activity of NE in cystic fibrosis lung secretions in the course of DNase therapy [7–10]. These studies gave, however, conflicting results some of which have been analysed in an editorial review by Vogelmeier and Döring [11]. Thus, it is not yet unambiguously established whether the DNase-promoted depolymerisation of DNA dissociates the in vivo formed DNA–NE complexes and thus increases the NE activity of airway secretions.

The present work was undertaken (i) to see whether DNA binds and inhibits cat G and PR3 in addition to NE and (ii) to study the inhibition of these proteinases by DNase-generated DNA fragments.

2. Materials and methods

2.1. Materials

NE and cat G were isolated and active site titrated as described previously [12]. PR3 was purchased from A.R.T. Athens, GA, USA. Mucus proteinase inhibitor was a gift from Novartis, Switzerland. DNase was from Hoffman-La Roche, Switzerland. Remazolbrilliant blue-labelled elastin came from E.P.C. Owenville, MO, USA. DNA-cellulose, proteinase K, RNase and chymostatin were from Sigma, St. Louis, MO, USA while Zincov came from Calbiochem-Novabiochem, San Diego, CA, USA. Buffer A is composed of 50 mM HEPES, 150 mM NaCl, pH 7.4. The lung secretions collected before and after inhalation of 2.5 mg of DNase were stored frozen.

2.2. Electrophoresis and chromatography

Lung secretions were incubated for 3 h at 50°C with 0.5% SDS, 100 μg/ml proteinase K, 20 μg/ml RNase and 5 mM EDTA. DNA was then extracted using phenol:chloroform:isoamylic alcohol (25:24:1) and precipitated with ethanol [13]. The pellet was dissolved in buffer A and kept frozen. Electrophoresis was carried out on a 0.5% agarose gel stained with ethidium bromide. A 500 bp ladder from Appligene, France, and a lambda DNA-*Hin*dIII digest from Amersham-Pharmacia, Sweden were used as size markers. Chromatography on DNA-cellulose was done as before [4].

2.3. Elastolytic activity

The elastolytic activity was measured at 37°C in buffer A as described previously [4]. Mixtures of proteinases and inhibitors were incubated for 15 min at 25°C before assay. Lung secretion extracts were prepared as follows: 1 g of frozen secretion was mixed with 1 ml of buffer A containing 1 mM Zincov, gently homogenised for 10 min at 0°C using a Potter device and centrifuged at $10\,000\times g$ during 30 min at 4°C. The elastolytic activity of the supernatants was then assayed in duplicate. Zincov, a potent metalloproteinase inhibitor [14] was used to inhibit zinc-containing metalloproteinases such as *Pseudomonas aeruginosa* elastase [15] and type IV collagenase [16,17] which may contribute to the elastolytic activity of lung secretions.

3. Results and discussion

3.1. Affinity chromatography on DNA-cellulose

At low ionic strength (50 mM HEPES, pH 7.4) NE and cat

*Corresponding author. Fax: (33)-3-88 67 92 42.
E-mail: jgbieth@pharma.u-strasbg.fr

Abbreviations: NE, neutrophil elastase; cat G, neutrophil cathepsin G; PR3, neutrophil proteinase 3

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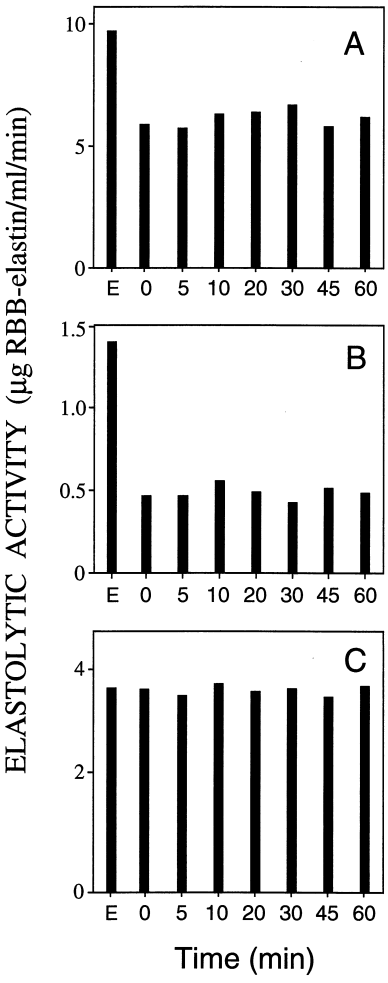

Fig. 1. Elastolytic activity of NE (A), cat G (B) and PR3 (C) in the presence of native DNA (time 0) and of DNA digested for variable periods of time with DNase. The digestion was done by incubating 2 mg/ml DNA with 0.2 µM DNase at 25°C in buffer A+1 mM $CaCl_2$+1 mM $MgCl_2$ [20]. After addition of 2.5 mM EDTA to stop the digestion, the fragmented DNA was mixed with proteinase and the elastolytic activity was measured. 'E' = NE, cat G or PR3 control without DNA. The final proteinase and DNA concentrations were 1 µM and 1 mg/ml, respectively. The elastolytic activities reported in the figure are the mean values resulting from triplicate runs. The standard errors (≤6%) were too small to be reported on the top of the activity bars.

G were bound on DNA-cellulose whereas PR3 was eluted unretarded from the column. A linear NaCl gradient eluted NE as a symmetrical peak whose top corresponded to 160 mM NaCl. Thus, with the physiological buffer used in this paper, the affinity of DNA for NE is low. In contrast, cat G bound so tightly to the column that it could not be eluted from it, indicating that it interacts very strongly with DNA. The fact that cat G, NE and PR3 have a decreasing order of affinity for DNA is in accordance with their decreasing cationic character as evidenced by electrophoresis [18].

3.2. *Effect of in vitro generated DNA fragments on the elastolytic activity of pure NE, cat G and PR3*

Fig. 1 shows that full length DNA partially inhibits the elastolytic activity of NE (37 ± 2% inhibition) and cat G (67 ± 2% inhibition) but has no effect on the elastolytic activity of PR3. When DNA was incubated with DNase for variable periods of time prior to its reaction with the proteinases, its proteinase inhibiting activity did not change (Fig. 1) although its size considerably decreased (< 0.5 kb, see Fig. 2). Low molecular mass DNA fragments are therefore as efficient as full length DNA in inhibiting NE and cat G.

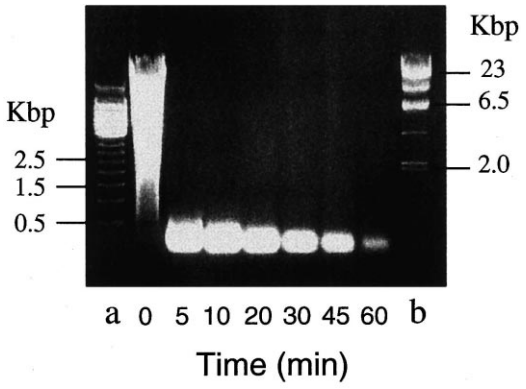

Fig. 2. Electrophoresis and ethidium bromide staining of DNA digested for variable periods of time with DNase and ethidium bromide staining. Lanes 1 and 9, molecular size markers: 500 bp ladder (lane 1) and lambda DNA-*Hin*dIII digest (lane 9). Lane 2: native DNA. Lanes 3–8: DNA digested for 5, 10, 20, 30, 45 and 60 min under the conditions given in the legend to Fig. 1.

3.3. *Effect of in vivo DNA fragmentation on the elastolytic activity of NE, cat G and PR3*

DNase therapy considerably depolymerises DNA in cystic fibrosis lung secretions, the average DNA size being about 0.4 kb [6]. We wanted to know whether the in vivo hydrolysis of DNA by DNase may partially release neutrophil proteinases from their nucleoprotein complexes. To this end, we designed a specific assay of NE, cat G and PR3 using elastin as a substrate in the absence or presence of mucus proteinase inhibitor, a protein that inhibits NE and cat G but not PR3 or chymostatin, a specific inhibitor of cat G [1]. Preliminary experiments were done with pure enzymes. Table 1 shows that indeed mucus proteinase inhibitor does not inhibit PR3 while chymostatin specifically inhibits cat G. On the other hand, the elastolytic activities of the mixtures (NE+cat G+PR3) ± inhibitor correspond to the sum of the activities of the individual enzymes ± inhibitor. Thus, the elastolytic activity of Zincov-containing lung secretions (see Section 2) in the absence and presence of mucus proteinase inhibitor or chymostatin allows separate measurement of the activity of NE, cat G and PR3.

Table 1
Elastolytic activity (µg elastin solubilised/ml/min) of 0.1 µM pure NE, cat G or PR3 in the absence or presence of 80 µM mucus proteinase inhibitor or 100 µM chymostatin

Inhibitors	Proteinases			
	NE	cat G	PR3	NE+cat G+PR3
None	0.97	0.14	0.36	1.49
Mucus proteinase inhibitor	0	0	0.33	0.37
Chymostatin	0.90	0	0.34	1.36

The figures are average values of 3–4 assays. The reproducibility of the technique was measured by repeating the same assay 6–8 times and calculating the relative coefficient of variation (standard deviation/mean × 100). This parameter was found to lie between 3 and 4%.

Table 2
Mean elastolytic activity of NE, cat G and PR3 in the lung secretions of 18 cystic fibrosis patients before and 30 min after DNase inhalation

	NE	cat G	PR3
Before DNase	52 ± 8	5.8 ± 0.8	5.2 ± 1.0
30 min after DNase	58 ± 12	5.1 ± 1.5	5.7 ± 1.1

The activities are expressed in μg elastin solubilised/ml/min. The figures are means ± S.E.M. The differences between before and after DNase were not significant (Wilcoxon signed rank test, $P > 0.9$).

The measurements were done on 18 lung secretions collected before and 30 min after DNase inhalation. There were no systematic increases but rather random variations in NE and cat G activities following DNase administration. PR3 which is not inhibited by DNA, underwent similar variations. Table 2 shows that these variations are not statistically significant. The lack of effect of DNase was confirmed by ex vivo experiments showing that the elastolytic activity of a number of cystic fibrosis lung secretions was not enhanced following 1 h reaction with 0.2 μM DNase, a concentration close to that found in lung secretions following DNase therapy [19]. Altogether, the above data confirm the in vitro observation that small DNA fragments inhibit NE and cat G as potently as full length DNA.

4. Conclusion

We conclude that DNA has a beneficial effect on the proteolytic potential of cystic fibrosis lung secretions since it blocks about 40% of the elastolytic activity of NE and 70% of that of cat G. This beneficial effect is not lost following DNase administration. PR3, which is not inhibited by DNA, might be more deleterious to the lung than the other two enzymes.

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