Effect of Polynucleotides on the Inhibition of Neutrophil Elastase by Mucus Proteinase Inhibitor and α_1-Proteinase Inhibitor†

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ABSTRACT: DNA released from neutrophils at sites of inflammation may modulate tissue proteolysis. We used tRNA and synthetic polynucleotides as models of DNA to study the influence of polynucleotides on the inhibition of neutrophil elastase by its endogenous inhibitors α_1-proteinase inhibitor (α_1-PI) and mucus proteinase inhibitor (MPI). Affinity chromatography showed that polynucleotides form electrostatic complexes with elastase and MPI but not with α_1-PI, the highest affinity being for MPI. The tight-binding partial inhibition of elastase by polynucleotides was used to calculate the K_d of the elastase−polynucleotide complexes which ranged from 4 μM to 21 nM. One mole of tRNA was able to bind 9 mol of elastase. Polydeoxycytosine and tRNA significantly impaired the reversible inhibition of elastase by MPI: they moderately increased the rate of enzyme−inhibitor association, strongly enhanced the rate of complex dissociation, and lowered the enzyme−inhibitor affinity by factors of 34 and 134, respectively. The two polynucleotides also decreased the rate of the irreversible inhibition of elastase by α_1-PI by factors of 30 and 3, respectively. Polynucleotides also changed the mechanism of inhibition of elastase by the two inhibitors from a one-step inhibition reaction to a two-step binding mechanism. Our data may help explain why proteolysis may occur at sites of inflammation despite the presence of active proteinase inhibitors.

Human polymorphonuclear leukocytes contain a number of proteolytic enzymes including neutrophil elastase (NE),[1] a 30-kDa glycoprotein that belongs to the class of serine proteinases. NE cleaves extracellular matrix proteins including elastin, interstitial collagen, proteoglycan, fibronectin, and laminin as well as plasma proteins such as antithrombin, fibrinogen, and components of the immune system. Uncontrolled release of this potent enzyme may lead to degenerative connective tissue diseases such as lung emphysema and rheumatoid arthritis (for a review, see ref 1).

The concentration of NE in the azurophil granules is thought to be in the millimolar range (*2*). An efficient anti-NE control system must therefore be present at sites where neutrophils are activated or where they die in order to prevent undesirable extracellular protein degradation. The anti-elastase control system is composed of three protein proteinase inhibitors: α_2-macroglobulin, α_1-proteinase inhibitor (α_1-PI), and mucus proteinase inhibitor (MPI). The two former proteins are present in plasma and in the interstitial fluid while the latter almost exclusively occurs in airways and genital tract secretions (for a review, see ref *1*).

The 53-kDa glycoprotein α_1-PI is thought to be the most important NE inhibitor. It belongs to the serine proteinase inhibitors, the serpin (*3*), a superfamily of proteins that have developed by divergent evolution over 500 million years. Many of the serpins are present in plasma and interstitial fluid where they inhibit irreversibly neutrophil coagulation, and complement serine proteinases by forming denaturant-stable complexes with them (*4*). Among all proteinases tested on α_1-PI, NE was found to react with the highest second-order association rate constant ($k_{assoc} \approx 10^7$ M^{-1} s^{-1}) (*5*−*8*). The high k_{assoc} value, the elevated levels of α_1-PI in plasma (~20 μM, see ref *9*) and in lung alveolar epithelial lining fluid (~4 μM, see ref *10*), and the pseudo-irreversible character of the binding of NE to α_1-PI (*5*, *7*, *8*, *11*) render the inhibition process extremely fast and efficient. For example, the delay time of inhibition (*12*), that is, the time required to fully inhibit NE in vivo, is only 13 ms in plasma (*1*) and 100 ms in the lung epithelial lining fluid (*10*). The anti-NE function of α_1-PI is best illustrated by the well-known correlation between hereditary α_1-PI deficiency and pulmonary emphysema (*1*).

MPI is the most abundant physiologic NE inhibitor of the upper respiratory tract, where it occurs in concentrations as high as 5 μM (*13*, *14*). It is a 11.7-kDa basic protein whose structure is stabilized by eight disulfide bonds. It is formed of a single chain of 107 amino acid residues of known sequence (*15*, *16*) and is composed of two domains of similar size and architecture (*17*). The X-ray diffraction study on

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[1] Abbreviations: NE, human neutrophil elastase; MPI, mucus proteinase inhibitor = secretory leukoprotease inhibitor (SLPI); polydN, polydeoxynucleotides (N = adenosin (A), cytidin (C), thymidin (T), or guanosin (G)), for example polydC(24−36) = polydeoxycytidin with 24−36 nucleotides; α_1-PI, α_1-proteinase inhibitor = α_1-antitrypsin; Suc-Ala$_3$-*p*NA, succinyl-Ala-Ala-Ala-*p*-nitroanilide; MeOSuc-Ala$_2$-Pro-Val-pNA, methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide; MeOSuc-Ala$_2$-Pro-Ala-SBzl, methoxysuccinyl-Ala-Ala-Pro-Ala-thiobenzylester.

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the MPI−chymotrypsin complex has identified Leu[72] as the P1 residue of the inhibitor's active site. NE binds at the same site as chymotrypsin (*18*).

In vivo, the inhibition of NE by MPI and α_1-PI may be influenced by compounds present at the site where the inhibition reaction takes place. For example, polymorphonuclear neutrophils present at sites of inflammation may release oxidants which may lower the antielastase activity of α_1-PI (*19*) and MPI (*20*). On the other hand, when heparin, an anionic polymer, is administered in some patients, it will bind NE and decrease its rate of inhibition by α_1-PI (*8*). Heparin may also have a positive effect on the inhibition of NE since it tightly binds MPI and so accelerates the inhibition of the enzyme. Furthermore, tissue inflammation is usually accompanied by a recruitment of neutrophils. When these short-lived cells die, they release not only their granule proteinases but also their nuclear DNA content. In the lung inflammation that characterizes cystic fibrosis, the DNA content of bronchial secretions may be larger than 3 mg/mL (*21*). Like heparin, the polyanionic DNA molecule binds both NE and MPI. Using fast kinetic analysis of inhibition, we have shown that, at the highest concentration that could be used, DNA decreased the enzyme−inhibitor affinity by a factor of 160 (*22*). Independently, Ying and Simon (*23*) using ordinary kinetic analysis showed that DNA increased the enzyme−inhibitor affinity by 2 orders of magnitude.

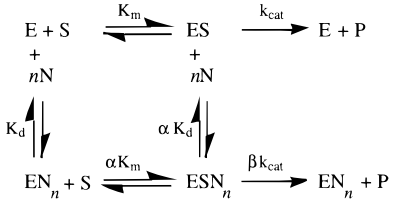
Kinetic investigations with full-length DNA are hampered by DNA solubility problems and reproducibility of the assays. In our previous investigation, the highest soluble DNA concentration was 200 μg/mL. At this concentration the maximal effects of DNA on the NE−MPI association were not yet observed. We therefore decided to use tRNA and synthetic polynucleotides as models of DNA. These compounds were tested on both MPI and α_1-PI, the two major physiological inhibitors of NE.

EXPERIMENTAL PROCEDURES

Materials. Human NE was isolated and active site titrated as described previously (*22*). Human recombinant MPI (from Synergen, Boulder, CO) and α_1-PI were obtained through the courtesy of Dr. H. P. Schnebli, Novartis, Switzerland. tRNA was from Boehringer (Germany) while the polydN were from Sigma. Suc-Ala$_3$-*p*NA and MeOSuc-Ala$_2$-Pro-Val-*p*NA were from Bachem, while MeOSuc-Ala$_2$-Pro-Ala-SBzl came from Enzyme Systems Products, Livermore, CA. Stock solutions of most substrates and of 4,4′-dithiodipyridine (Sigma) were made in dimethylformamide, while Suc-Ala$_3$-*p*NA was dissolved in *N*-methylpyrrolidone. The final concentration of organic solvents was 2% (v/v) throughout. Unless otherwise stated, all experiments were performed at 25 °C in 50 mM Hepes, 100 mM NaCl, pH 7.4. NE, MPI, and α_1-PI were coupled to epoxy-activated Sepharose from Pharmacia, as described by the manufacturer. The affinity supports were poured into HR 10/10 Pharmacia columns and equilibrated with 50 mM Hepes buffer, pH 7.4.

Effect of Polynucleotides on the Inhibition of NE by MPI and α_1-PI. The rate of inhibition of NE by MPI or α_1-PI was measured under pseudo-first-order conditions, that is, $[I]_o \geq 10[E]_0$, using the progress curve method which consists of adding enzyme to a mixture of inhibitor and substrate and recording the release of product as a function of time (*24*−*28*). NE with or without polynucleotide was added to a buffered solution containing MPI, 0.3 mM MeOSuc-Ala$_2$-Pro-Ala-SBzl, and 3 mM 4,4′-dithiodipyridine or to a buffered solution of α_1-PI and 2 mM MeOSuc-Ala$_2$-Pro-Val-*p*NA. A SF/PQ 53 stopped-flow apparatus (Hi-Tech Scientific, Salisbury, UK) with a dead time of about 1 ms was used to mix the reagents and to record and analyze the progress curves.

Scheme 1



Equilibrium Dissociation Constant K_d of the NE−Polynucleotide Complexes. tRNA and some of the polydN form complexes with NE that are partially active on synthetic substrates. This type of inhibition may be described by Scheme 1 where E, S, and N stand for enzyme, substrate, and polynucleotide, respectively, *n* is the number of enzyme molecules bound per molecule of polynucleotide, and α and β are dimensionless numbers. The conditions under which partial inhibition takes place may be summarized by $0 < \beta \leq 1$ and/or $\infty > \alpha \geq 1$. In case of tight binding of E to N, the steady-state rate equation corresponding to Scheme 1 is given by the following (*29*):

$$\frac{v_i}{v_0} = \frac{v_0 - v_\infty}{2v_0}\left(\left[\left(A + \frac{[N]_0}{n[E]_0} - 1\right)^2 + 4A\right]^{1/2} + \frac{v_0 + v_\infty}{v_0 - v_\infty} - A - \frac{[N]_0}{n[E]_0}\right) \quad (1)$$

with

$$A = \frac{1 + [S]_0/K_m}{\alpha + [S]_0/K_m}\frac{\alpha K_d}{[E]_0} \quad (1a)$$

and

$$v_\infty = \beta\frac{k_{cat}[E]_0}{[S]_0 + \alpha K_m} \quad (1b)$$

where v_i/v_0 = enzymatic rate in the presence of polynucleotide/enzymatic rate in the absence of polynucleotide, v_∞ = limit of v_i for saturating concentrations of polynucleotide, and $[E]_0$ and $[N]_0$ are the total concentrations of enzyme and polynucleotide, respectively.

The fact that eqs 1 and 1b contain six unknown parameters (K_d, k_{cat}, K_m, α, β, and *n*) renders the nonlinear regression analysis impossible to perform. Separate experiments were therefore run to measure k_{cat}, K_m, α, β, and *n*. To this end we determinated k_{cat} and K_m, the kinetic parameters describing the hydrolysis of substrate by free NE, and βk_{cat} and αK_m, the parameters for the hydrolysis of substrate by NE in the presence of a saturating concentration of polynucleotide. The NE−tRNA binding stoichiometry *n* was measured by incubating constant concentrations of NE (2.5 μM) with increasing concentrations of tRNA in 50 mM Hepes buffer,

Table 1: Affinity Chromatography of Polynucleotides on Sepharose−NE or Sepharose−MPI Columns[a]

nucleotides	Sepharose−NE [NaCl] (mM)	Sepharose−MPI [NaCl] (mM)
tRNA	150	190
polydA(12−18)	59	80
polydA(25−30)	76	113
polydT(12−18)	97	162
polydT(25−30)	181	201
polydC(12−18)	152	181
polydC(24−36)	201	210
polydG(12−18)	191	249

[a] The columns were equilibrated with 50 mM Hepes, pH 7.4. The polynucleotides were dissolved in the same buffer and eluted from the columns with a linear NaCl gradient at a flow rate of 0.5 mL min^{-1}. The NaCl concentration corresponding to the top of the elution peak is indicated.

pH 7.4, without NaCl and measuring the residual NE activity with elastin (*30*). Under these conditions, the tRNA is a tight-binding NE inhibitor which yields a straight titration curve from which $n = 9$ was derived. The binding stoichiometries for the complexes of NE with the other polynucleotides were assumed to be $n \approx 1$ as derived from the tangents of the v_i/v_o versus [polydN] plots. When these plots were analyzed using K_d and n as independent variables, the best fits were always obtained with $n = 1$. The parameters k_{cat}, K_m, α, β, and n were used as known constants of eq 1 so that K_d could be determined from nonlinear iterations based on K_d only.

RESULTS

Affinity Chromatography To Assess the Binding of Polynucleotides with NE, MPI, and α_1-PI. The capacity of polynucleotides to bind NE was investigated using a Sepharose−NE affinity column. Table 1 shows that tRNA elutes from the column with 150 mM NaCl while the elution of the polydN depends on their structure and size: the higher the latter, the better the affinity. For a given size, the best interaction is with polydG, followed by polydC and polydT. The interaction between NE and polydA is negligible above 100 mM NaCl.

tRNA was eluted at 190 mM NaCl from Sepharose−MPI, a concentration higher than that necessary for its elution from Sepharose−NE. Higher salt concentrations were also required to elute the polydN from Sepharose-MPI (Table 1). With this affinity support, all of the nucleotides except polydA(12−18) were eluted at a concentration of NaCl greater than 100 mM. As with NE, both the size and the nature of the nucleotide are important (Table 1).

Neither tRNA nor the polydN tested were able to bind to Sepharose−α_1-PI.

Determination of the Equilibrium Dissociation Constants K_d of the NE−Polynucleotide Complexes. The equilibrium dissociation constants were determined by reacting constant concentrations of NE with increasing concentrations of polynucleotides and measuring the residual enzyme activities with a synthetic substrate. Figure 1 shows, for example, the effect of polydC(24−36) on the activity of NE. A K_d of 30 nM was calculated from these partial inhibition data as indicated in the legend to Figure 1 and in the Experimental Section. The K_d values for other polynucleotide−NE complexes could also be determined in this way (Table 2).

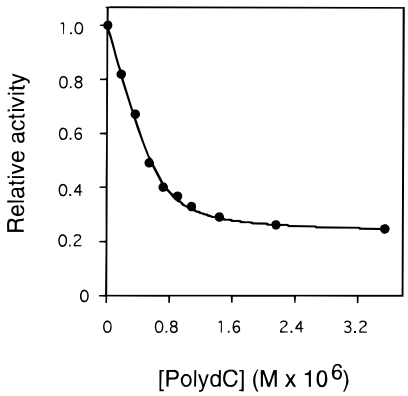


FIGURE 1: Determination of the equilibrium dissociation constant of the NE−polydC(24−36) complex. The relative activity was measured using constant concentrations of NE (0.67 μM) and Suc-Ala_3-*p*NA (1 mM) and variable concentrations of polydC(24−36). The data were fit to eq 1 by nonlinear regression analysis. The fitting procedure used K_d as the only dependent variable, K_m, k_{cat}, α, β, and n being determined in separate experiments.

Table 2: Equilibrium Dissociation Constants K_d of Some NE−Polynucleotide Complexes at pH 7.4 and 25 °C[a]

nucleotides	K_d (M)
tRNA (no NaCl)	1.5×10^{-9}
tRNA (50 mM NaCl)	6.3×10^{-9}
tRNA (100 mM NaCl)	2.1×10^{-8}
polydA(12−18)	*b*
polydA(25−30)	*b*
polydT(12−18)	*b*
polydT(25−30)	2.0×10^{-7}
polydC(12−18)	4.0×10^{-6}
polydC(24−36)	3.0×10^{-8}
polydG(12−18)	3.0×10^{-7}

[a] The NaCl concentration was 100 mM unless otherwise stated. In the case of tRNA, K_d was measured using constant concentrations of NE (50 nM) and MeOSuc-Ala_2-Pro-Val-*p*NA (1 mM) and variable concentrations of tRNA. The other K_d values were determinated as described for polydC(24−36) in the legend to Figure 1. The errors on K_d are less than 15% and are not given. [b] No inhibition of NE.

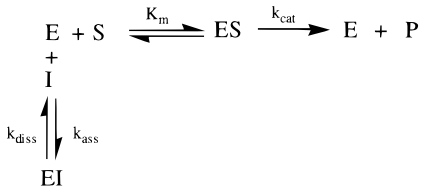
The dissociation constant for the NE−tRNA complex was found to be ionic-strength-dependent, in agreement with the salt-dependent elution of tRNA from the Sepharose−NE column. The tight NE−tRNA binding observed at $\mu = 0$ ($K_d = 1.5$ nM) explains why a straight inhibition curve was obtained upon reacting 2.5 μM NE with increasing concentrations of tRNA (see Experimental Section).

Inhibition of NE by MPI in the Presence of Polynucleotides. To study the rate of inhibition of NE by MPI in the presence of polynucleotides, we used the progress curve method outlined in the Experimental Section. Since the inhibitor was tested under pseudo-first-order conditions and the substrate was not depleted to a significant extent during the reaction, the progress curves may be described by the following equation (*24*−*26*):

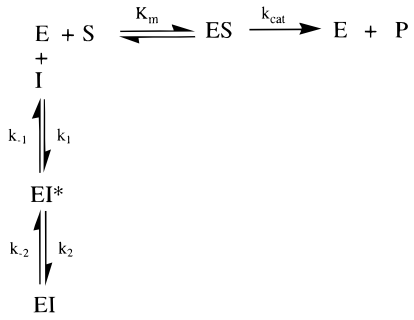
$$[P] = v_s t + \frac{v_z - v_s}{k}(1 - e^{-kt}) \qquad (2)$$

where [P] is the product concentration, v_z is the velocity at $t = 0$, v_s is the steady-state velocity, and k is the apparent first-order rate constant for the approach to steady state. The progress curves were analyzed by nonlinear regression to calculate k. The reversible competitive inhibition process

Scheme 2



Scheme 3



may be a one-step or a two-step reaction illustrated by Schemes 2 and 3 where E stands for NE or the polynucleotide−NE complex, I represents MPI or the polynucleotide−MPI complex, EI is the final stable complex, and EI* is a rapidly formed intermediate whose concentration is governed by $K_i^* = k_{-1}/k_1$. Schemes 2 and 3 predict eq 3 and 4, respectively:

$$k = \frac{k_{ass}[I]_0}{1 + [S]_0/K_m} + k_{diss} \quad (3)$$

$$k = \frac{k_2[I]_0}{[I]_0 + K_i^*(1 + [S]_0/K_m)} + k_{-2} \quad (4)$$

To determine whether Scheme 2 or Scheme 3 accounts for the inhibition of the NE−tRNA complex by the MPI−tRNA complex, the apparent rate constant k was measured as a function of the MPI concentration in the presence of a constant concentration of NE, tRNA, and substrate. We used a 2 μM tRNA concentration which saturates NE to >99%. The estimated saturation of MPI was at least equal to that of NE since the NaCl concentration needed to elute tRNA from the Sepharose−MPI was larger than that needed to elute it from the Sepharose−NE column (Table 1). The apparent rate constant k varied hyperbolically with the MPI concentration (Figure 2), suggesting that the inhibition of NE by MPI in the presence of tRNA is best described by a two-step interaction model (Scheme 3). Indeed, the data could be fit to eq 4 by nonlinear regression analysis. We found $K_i^* = 0.27$ μM, $k_2 = 2.6$ s^{-1}, and $k_{-2} = 0.04$ s^{-1}. The equilibrium dissociation constant of the final EI complex given by $K_i = K_i^* k_{-2}/(k_2 + k_{-2})$ was found to be 4.3 nM.

The inhibition of NE by MPI in the presence of polynucleotides was studied using either polydC(24−36), which binds both NE and MPI at 100 mM NaCl, or polydT(12−18), which binds MPI (Table 1) but not NE (Table 2). To choose the concentration of polydC(24−36), we have measured k with constant concentrations of NE, MPI, and substrate and variable concentrations of polydC(24−36). The rate constant k increased and then reached a plateau for a polydC(24−36) concentration of 4 μM. At this concentration, both NE and MPI were, therefore, assumed to be saturated with polynucleotide. We then measured k as a function of MPI concentration and found it to be linearly related to the inhibitor concentration, indicating a one-step inhibition (Scheme 2). Linear regression analysis yielded $k_{ass} = 10^7$ M^{-1} s^{-1} and $k_{diss} = 1.1 \times 10^{-2}$ s^{-1}. The calculated K_i ($= k_{diss}/k_{ass}$) was found to be 1.1 nM. Using polydT(12−18) at the same concentration as polydC(24−36), k was again found to be linearly related to the MPI concentration and k_{ass}, k_{diss}, and K_i were found to be 4.8×10^6 M^{-1} s^{-1}, 1.1×10^{-3} s^{-1}, and 0.23 nM, respectively. The kinetic constants for the inhibition of NE by MPI in the presence of polynucleotides are compiled in Table 3.

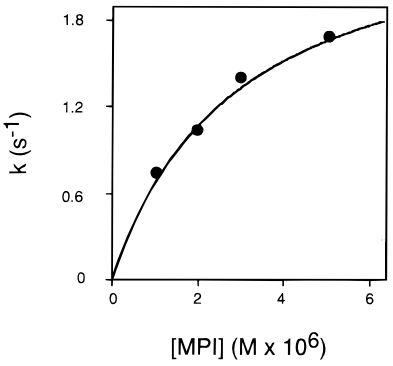


FIGURE 2: Effect of MPI on k, the pseudo-first-order rate constant of NE inhibition in the presence of 2 μM tRNA and 0.1 μM NE. The theoretical curve was calculated using eq 4 and the best estimates of K_i^*, k_{-2}, and k_2.

Inhibition of NE by α1-PI in the Presence of Polynucleotides. The inhibition of NE by α_1-PI in the absence or presence of polynucleotides was also studied using the progress curve method. With α_1-PI the inhibition is irreversible (*5*, *31*), so that the progress curves may be described by eq 5 (*27*):

$$[P] = \frac{v_z}{k}(1 - e^{-kt}) \quad (5)$$

The equation for one-step inhibition (Scheme 2 with $k_{diss} = 0$) or two-step inhibition (Scheme 3 with $k_{-2} = 0$) will then be the following:

$$k = \frac{k_{ass}[I]_0}{1 + [S]_0/K_m} \quad (6)$$

$$k = \frac{k_2[I]_0}{[I]_0 + K_i^*(1 + [S]_0/K_m)} \quad (7)$$

In the absence of tRNA, the apparent rate constant k increased linearly with the α_1-PI concentration up to 6 μM, the highest inhibitor concentration compatible with a reliable measurement of the rate constant (Figure 3A). Therefore, within this concentration range, the mechanism of inhibition of NE by α_1-PI is consistent with a one-step reaction. A k_{ass} value of 6.5×10^6 M^{-1} s^{-1} was calculated by linear regression analysis of the data. In the presence of 0.1 μM tRNA which saturates NE to the extent of 99%, the apparent rate constant k varied hyperbolically with the α_1-PI concentration (Figure 3B), indicating that the inhibition is a two-step process. Indeed, the data could be fit to eq 7 by

Table 3: Kinetic Constants Characterizing the Inhibition of NE by MPI in the Absence or Presence of tRNA, polydC(24−36), and polydT(12−18) at pH 7.4, 100 mM NaCl and 25 °C[a]

polynucleotides	inhibition mechanism	K_i^* (M)	k_2 (s^{-1})	k_2/K_i^* or k_{ass} (M^{-1} s^{-1})	k_{-2} or k_{diss} (s^{-1})	K_i (M)
none[b]	one-step	$>2 \times 10^{-6}$	>6	3.1×10^6	1.0×10^{-4}	3.2×10^{-11}
tRNA	two-step	2.7×10^{-7}	2.6	9.2×10^6	4.0×10^{-2}	4.3×10^{-9}
polydC(24−36)	one-step			1.0×10^7	1.1×10^{-2}	1.1×10^{-9}
polydT(12−18)	one-step			4.8×10^6	1.1×10^{-3}	2.3×10^{-10}

[a] The errors on the kinetic parameters are less than 15% (experimental constants) or 30% (calculated constants) and are not reported. [b] From Cadène et al. (*34*).

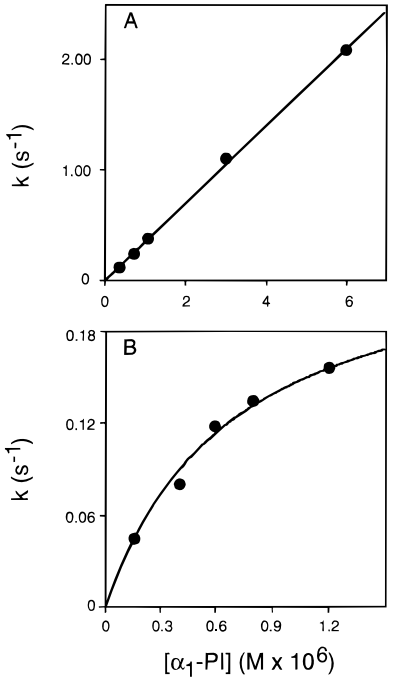


FIGURE 3: Effect of α_1-PI on *k*, the pseudo-first-order rate constant of NE inhibition in the absence (panel A) or presence (panel B) of 0.1 μM tRNA. The experiments were done using increasing concentrations of α_1-PI and NE and [α_1-PI] ≥ 10 [NE]. The curves are theoretical and have been calculated using eq 6 (panel A) or eq 7 (panel B) and the best estimates of the kinetic constants.

nonlinear regression analysis which yielded the best estimates of K_i* (0.14 μM) and k_2 (0.27 s^{-1}). In the presence of a saturating concentration of polydC(24−36) the inhibition was also a two-step reaction described by $K_i^* = 0.38$ μM and $k_2 = 0.08$ s^{-1}. In contrast, polydT(12−18) which does not bind NE (Table 2) did not change the inhibition mechanism at a concentration as high as 7 μM and yielded $k_{ass} = 3.7 \times 10^6$ M^{-1} s^{-1}, a value close to that found in the absence of the polynucleotide. The whole set of constants describing the inhibition of NE by α_1-PI is given in Table 4.

DISCUSSION

We have used tRNA and a number of synthetic polydeoxynucleotides as models of DNA to study the effect of polynucleotides on the inhibition of NE by MPI and α_1-PI in vitro and to see whether DNA may affect the inhibition of NE in vivo. Affinity chromatography suggests that the binding of polynucleotides with NE and MPI is electrostatic in nature and probably involves ionic interactions between some basic amino acid residues of the two cationic proteins and some phospho-(deoxy)ribosyl moieties of the anionic polymers. The deleterious effect of ionic strength on the K_d of the elastase−tRNA complex (Table 2), as well as the fact that the larger the size of the polydeoxynucleotides, the better their affinity for NE, confirms the occurrence of ionic interactions. Affinity chromatography also suggests that polynucleotides have a better affinity for MPI than for NE. The former protein has 15 Lys and 5 Arg residues, that is, 20 positive charges per $M_r = 11\,700$ (*32*), while NE has 17 fully exposed Arg residues, that is, 17 positive charges per $M_r = 30\,000$ (*33*). These charge differences may account for the difference in the affinity of the two proteins for the various polynucleotides. Heparin, another anionic polymer, also tightly binds NE and MPI (*34*). However, unlike polynucleotides, this sulfated glycosaminoglycan has a much better affinity for NE than for MPI (*34*). Perhaps the polynucleotide−NE binding is mostly electrostatic while the heparin−NE interaction involves little ionic interactions, the binding energy being mainly due to nonionic interactions as recently shown for the neutrophil cathepsin G-heparin interaction (*35*). Due to these nonionic interactions heparin may be a more specific NE ligand than polynucleotides. The polynucleotides studied in this work do not bind α_1-PI, a property shared by heparin (*8*).

Table 4: Kinetic Constants Characterizing the Inhibition of NE by α_1-PI in the Absence and Presence of tRNA, polydC(24−36), and polydT(12−18) at pH 7.4, 100 mM NaCl, 25 °C[a]

polynucleotides	inhibition mechanism	K_i^* (M)	k_2 (s^{-1})	k_2/K_i^* or k_{assoc} (M^{-1} s^{-1})
none[b]	one-step[b]	$>4 \times 10^{-7}$	>3	6.5×10^6
tRNA	two-step	1.4×10^{-7}	0.27	1.9×10^6
polydC(24−36)	two-step	3.8×10^{-7}	0.08	2.1×10^5
polydT(12−18)	one-step			3.7×10^6

[a] The errors on the kinetic parameters are less than 15% (experimental constants) or 30% (calculated constants) and are not reported. [b] The lower limits of K_i^* and k_2 were calculated assuming that the inhibition is a two-step process whose $K_i^*(1 + [S]_o/K_m)$ is greater than the highest inhibitor concentration used (see also text).

Like heparin (*8*), the polynucleotides are partial inhibitors of NE (e.g., Figure 1). The inhibition data could be satisfactorily fitted to eq 1, indicating that they are described by Scheme 1 which assumes that the inhibition potency depends on *n*, the polynucleotide−NE binding stoichiometry, K_d, the equilibrium dissociation constant of the NE−polynucleotide complex, and the two dimensionless numbers α and β. Theoretically, incomplete inhibition as observed here occurs if $0 < \beta \leq 1$ and/or $1 \leq \alpha < \infty$ (see eq 1b). We have measured α and β for all NE−polynucleotide systems for which enzyme inhibition was observed. These data (not shown) allow us to conclude that in all cases the partial inhibition is due to the combined effects of $\alpha > 1$ and $\beta < 1$. Use was made of the polynucleotide-induced inhibition of NE activity to calculate the K_d values of the NE−polynucleotide complexes.

Free NE and MPI react with each other according to a simple bimolecular reaction (*34*). In contrast, tRNA-bound MPI inhibits tRNA-bound NE via a two-step reaction mechanism where EI, the final inhibitory complex is preceded by EI*, a fast-forming intermediate whose concentration is governed by K_i^*. Since the k_{ass} for the inhibition of free NE by free MPI is 2−3 orders of magnitude lower than the maximum rate constant of a diffusion-controlled reaction, it has been assumed previously that this inhibitory reaction also involves an EI* intermediate even if the latter is not seen kinetically (*34*). The highest inhibitor concentration used in the previous experiments gave the lowest limits of K_i^* and k_2 reported in Table 3. By using this assumption, we may conclude that tRNA favors the initial binding of NE with MPI by significantly lowering K_i^* but impairs their rate of association by decreasing k_2. The overall effect of tRNA is, however, a ∼3-fold increase in the second-order association rate constant. The two other polynucleotides also moderately accelerate the inhibition of NE by MPI. tRNA and polydC(24−36) which bind both NE and MPI (Table 1) greatly destabilize the enzyme−inhibitor complex by dramatically increasing k_{-2} or k_{diss} (Table 3). In contrast, polydT(12−18), which strongly binds MPI but weakly interacts with NE (Tables 1 and 2), is much less deleterious on k_{diss} (Table 3). This suggests that the polynucleotides favor the dissociation of EI by creating steric hindrance to the tight protein−protein interaction. The effects of polynucleotides on the NE−MPI system are qualitatively similar but quantitatively different from those observed with heparin. This ligand increases k_{ass} about 10-fold but increases k_{diss} to an identical extent so that K_i is unchanged (*34*). Heparin therefore favors the inhibition of NE by MPI by accelerating the binding of the two proteins while polynucleotides impair this inhibition by destabilizing the final enzyme−inhibitor complex.

The inhibition of free NE by recombinant unglycosylated α_1-PI was found to be a one-step process (Figure 3A), a behavior identical to that observed previously with natural α_1-PI, the recombinant and the natural protein having similar association rate constants (*31*). The NE−tRNA and the NE−polydC(24−36) complexes react 3- and 30-fold slower with α_1-PI than with the free enzyme (Table 4), and the inhibition is a two-step reaction. In contrast, in the presence of polydT-(12−18) which weakly binds NE (Table 1), there is one-step inhibition with little alteration of k_{ass} (Table 4). This confirms that polynucleotides depress the k_{ass} of the NE + α_1-PI association by reacting with the enzyme. As with MPI, we may assume that the inhibition of free NE by α_1-PI is a two-step reaction with $K_i^* > 0.4\ \mu M$ and $k_2 > 3\ s^{-1}$. We may then conclude that tRNA and polydC(24−36) increase the enzyme−inhibitor affinity for the initial encounter complex EI* but sharply decrease the rate of conversion of EI* into EI, the global effect being a decrease in the second-order association rate constant k_2/K_i^*. The action of the polynucleotides is thus very similar to that of heparin which also promotes the buildup of a reaction intermediate EI* and decreases k_2 (*31*).

We believe that our kinetic data have pathological bearing. MPI and α_1-PI are the major NE inhibitors of the lung (*1*). Following their recruitment during lung inflammation, neutrophils may degranulate and release NE. In addition, when these short-lived cells die in situ, they also release their DNA (*36*). As may be inferred from our studies with model compounds, DNA may interfere with the in vivo inhibition of NE by MPI and α_1-PI. By dramatically increasing the dissociation rate constant of the NE−MPI complex, DNA will facilitate the dissociation of this complex by lung extracellular matrix proteins. Hence, lung tissue proteolysis may take place despite the presence of MPI. The action of DNA on the antielastase function of α_1-PI is more diffficult to predict. Since DNA decreases the rate of inhibition of NE by this irreversible inhibitor, it will increase the amount of matrix proteins turned over during the inhibition process. This deleterious effect might, however, be compensated for by a decrease in protein breakdown due to the partial inhibition of NE activity following binding of DNA. Experiments with NE, α_1-PI, DNA, and matrix proteins may answer the question of whether DNA favors in vivo proteolysis or not.

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