Hypothesis

Application of linear free energy relationships to the serpin–proteinase inhibition mechanism

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Received 21 March 2000; received in revised form 9 May 2000

Edited by Matti Saraste

Abstract Linear free energy relationships can be used to link the changes in rate constant for a reaction to changes in the equilibrium caused by alterations in structure. While they have most often been used in the analysis of chemical reactions, they have also been employed to resolve questions in enzymology and protein folding. Here we analyze the reaction of a serpin with a panel of six serine proteinases, and observe that a linear free energy relationship exists between the true second-order rate constant for reaction, k_{inh}, and the inhibition constant, K_I, indicating that formation of the covalent serpin–enzyme complex may be reversible.

Key words: Inhibition kinetics; LFER; Protease; Proteinaceous inhibitor; Poxvirus; Structure–activity relationship

1. Introduction

Serine proteinase inhibitors of the serpin family are structurally homologous proteins whose members play central roles in the regulation of a wide variety of physiological processes including fibrinolysis, coagulation, inflammation, fertilization, malignancy, neuromuscular patterning and development [1]. While most in the field now agree on a basic kinetic and mechanistic model [2], certain details of the mechanism by which serpins inhibit their target proteinases remain controversial. A powerful tool in the search to understand the relationship between molecular structure and activity is the linear free energy relationship (LFER). LFERs link the effect of a change in structure on the rate constant for a given reaction to its effect on the equilibrium constant for the same process [3]. In so doing, LFERs can shed light on the structure of the transition state for reaction relative to the reactant and product ground states, and on the role that specific interactions play in transition state stabilization. LFERs have primarily been used in the analysis of chemical reactions, but have increasingly found applications in enzymology, for example in probing the role of the catalytic lysine in aspartate aminotransferase [4,5], and in ribulose 1,5-bisphosphate carboxylase/oxygenase [6], in determining the mechanism of GTP hydrolysis by GTP-binding proteins [7–9], in the evaluation of the reaction mechanism of cytochrome P450 [10–12], and of energy coupling in the F_0F_1-ATP synthase reaction [13]. LFERs have also proven to be an invaluable tool in the analysis of protein folding [14], suggesting that they might be applicable to the serpin–proteinase inhibition mechanism in which the rate-limiting step is believed to involve a substantial conformational rearrangement of the serpin structure [2]. However, to date there has been no example of the application of LFERs to the serpin mechanism. In the analysis of the serpin inhibition mechanism, LFERs may offer a unique glimpse at events occurring in the transition states of key steps in the reaction, and may thus help resolve questions concerning the nature of these steps. Here we interpret data for the reaction of the secreted viral serpin SERP-1 with a panel of six serine proteinases in terms of the interactions that exist between serpin and enzyme at different points along the free energy profile for reaction. We also present the first demonstration that a linear free energy relationship exists between the true second-order rate constant for reaction, k_{inh}, and the inhibition constant, K_I. This finding lends support to proposals that the formation of the covalent inhibited serpin–enzyme complex may be reversible.

2. The serpin mechanism

The mechanism for the reaction of a serpin with a target proteinase, leading to the formation of a covalent inhibited serpin–proteinase complex, EI*, is incompletely understood. After extensive study by many groups, the branched reaction scheme shown in Scheme 1A has become accepted as a minimal kinetic mechanism for the reaction [2]. However, the precise natures of several of the steps in the reaction remain controversial. In Scheme 1A, enzyme (E) and serpin (I) initially interact to form a non-covalent Michaelis complex, EI, in which the reactive center loop (RCL) of the serpin occupies the active site cleft of the proteinase. The Michaelis complex then undergoes a reaction involving the catalytic functionality at the active site of the proteinase, leading to the formation of a covalent acyl–enzyme intermediate, [EI], in which the cleaved portion of the RCL has likely become partly inserted

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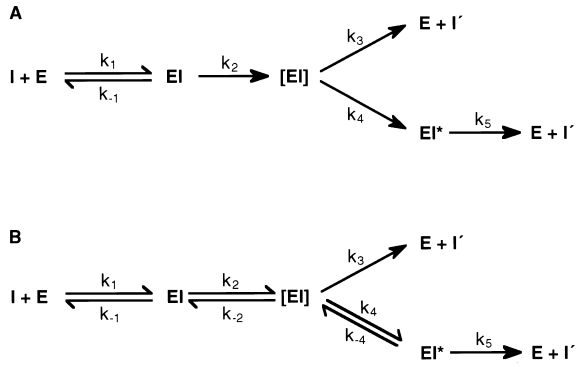
0014-5793/00/$20.00
PII: S0014-5793(00)01620-3



Scheme 1. A: Minimal kinetic scheme for the reaction of a serpin, I, with a proteinase, E, to form a stable, covalent inhibited complex, EI* (adapted from [2]). Initial formation of the non-covalent Michaelis complex, EI, is followed by acylation of the enzyme by the serpin to form the initial acyl–enzyme complex, [EI]. The intermediate complex [EI] can partition to undergo a conformational rearrangement resulting in the formation of the final inhibited complex, EI*, or to undergo hydrolytic deacylation to form cleaved serpin (I′) and release active enzyme, through the 'substrate pathway'. The final inhibited complex, EI*, slowly decomposes through a single pathway, resulting in the release of cleaved serpin and active enzyme with rate constant k_5. B: Modified kinetic scheme which incorporates the possibility that the steps with rate constants k_2 and k_4 might be reversible, and thus that EI* can decompose either through direct hydrolysis (with rate constant k_5), or by the reverse of its formation, through reversion to [EI]. In a case where active serpin can be quantitatively recovered from the complex, it must be true that $k_{-4} \gg k_5$, and $k_{-2} \gg k_3$ (see text).

into β-sheet A of the serpin structure. The intermediate [EI] can partition to react through two distinct pathways. It can undergo a conformational rearrangement involving insertion of the remainder of the cleaved RCL into β-sheet A [2], resulting in a substantial increase in stability of the serpin structure. This conformational change leads to the final inhibited complex, EI*, in which the tethered proteinase has moved to the opposite pole of the serpin where it interacts with the serpin structure in such a way that the catalytic machinery of the enzyme is forced to adopt an inactive conformation. Alternatively, the intermediate [EI] can undergo hydrolysis, catalyzed by the proteinase, leading to its deacylation in a step that resembles the reaction of a proteinase substrate. The products of deacylation are cleaved serpin, I′, and active proteinase. The final inhibited complex, EI*, can also undergo very slow hydrolysis to release active enzyme and cleaved serpin (Scheme 1A). Despite broad acceptance of the minimal kinetic scheme shown in Scheme 1A, numerous details of the mechanism remain the subjects of uncertainty and debate. For example, the degree to which the RCL becomes inserted into β-sheet A prior to RCL cleavage and acyl–enzyme formation is disputed, with one recent report suggesting that full insertion precedes the acylation that leads to the first covalent intermediate [15]. Similarly, one report has suggested that the final inhibited complex EI* may have a structure at the enzyme active site that corresponds to the tetrahedral intermediate that precedes acyl–enzyme formation in the serine proteinase reaction mechanism [16]. Another contentious issue concerns the degree to which formation of [EI] and its conversion to the final inhibited complex, EI*, is reversible. Several reports indicate that uncleaved, reactive serpin can be recovered from the EI* complex in at least some cases [16–18], indicating that in these cases all the steps leading to the formation of EI* must be reversible. Nevertheless, most reports show these steps as irreversible [2,20]. If the formation of EI* is reversible, even if only very slowly, this raises the possibility that the slow hydrolysis of EI* to release cleaved serpin plus active enzyme might occur, in whole or in part, through the reversion of EI* to [EI] followed by the deacylation of [EI] via the substrate pathway. It is possible to draw a kinetic scheme that incorporates some of the additional mechanistic possibilities that are raised by these unresolved questions (Scheme 1B). According to this mechanism, all of the steps are potentially reversible, though the rate constants could still heavily bias the reaction in a forward direction.

While the kinetic and mechanistic models in Scheme 1 serve as an excellent basis for the analysis of serpin–proteinase interactions, it is difficult in practice to directly measure the kinetic constants for most of the individual microscopic steps in this mechanism. Instead, we are limited to measuring macroscopic rate constants that in most cases are composite functions of the microscopic rate constants in Scheme 1 [19,21]. For example, the apparent second-order rate constant for reaction, k_{app}, is a measure of the overall rate at which a serpin reacts to form the final inhibited complex, EI*. However, because of the branched nature of the reaction pathway, this number is not identical to the true second-order rate constant for the reaction between E and I, k_{inh}. This is because, if only a fraction of [EI] goes on to form EI*, due to partitioning through the substrate branch of the pathway (with rate constant k_4), then the rate constant for the formation of EI* will be smaller than that for the formation of [EI] by a factor of $(1+k_3/k_4)$ [21]. Similarly, the macroscopically observed inhibition constant, $K_{I(app)}$, reflects the steady state balance between all of the pathways for the formation of inhibited enzyme species EI, [EI] and EI* and all of the pathways for the regeneration of active enzyme, including via the substrate pathway. In order to determine the true balance between the formation and decomposition of the final inhibited complex, EI*, it is again necessary to divide $K_{I(app)}$ by a factor of $(1+k_3/k_4)$ to compensate for the alternative route to the regeneration of active enzyme that is provided by partitioning through the substrate branch of the pathway [21]. Fortunately, it is possible to directly determine the partitioning ratio, k_3/k_4, by measuring the stoichiometry of inhibition (SI), i.e. the number of equivalents of serpin required to fully inactivate the enzyme under conditions where $[I] \gg K_{I(app)}$ [21]. This is because the requirement for additional equivalents of serpin to achieve complete inhibition stems directly from the cleavage of a fixed fraction of the serpin by reaction through the substrate pathway. Finally, the rate constant for the decomposition of EI* to release active enzyme, k_{diss}, can be measured directly. However, in the context of the reversible kinetic mechanism in Scheme 1B, k_{diss} represents the sum of k_5+k_{-4} (at least for reactions where $SI > 2$; for reactions where $SI \leq 2$ k_{diss} is a more complex function of k_5, k_{-4} and other rate constants).

3. Observation of a linear free energy relationship for the reactions of SERP-1

An extensive data set, encompassing the reaction of a single serpin with a panel of six serine proteinases under identical conditions, is available for the myxoma virus serpin, SERP-1 [22]. SERP-1 is one of many immunomodulatory proteins

produced by myxoma virus [23], but has the distinction of being the only known virus-encoded secreted serpin. SERP-1 serves to dampen the in vivo inflammatory response to virus infection [24]. Purified SERP-1 protein has also been employed as an effective anti-inflammatory agent in model systems relevant to human disease [25,26]. Biochemically, SERP-1 has been shown to strongly inhibit enzymes of the plasminogen cascade such as tissue-type plasminogen activator (t-PA), urokinase (u-PA) and plasmin [22,27]. It is not clear yet whether these are in fact the biological targets for SERP-1 that are responsible for its activity in vivo. Values for k_{app}, $K_{I(app)}$, k_{diss}, SI, k_{inh} and K_I for the interaction of SERP-1 with urokinase (uPA), tissue-type plasminogen activator (tPA), plasmin, thrombin, factor Xa, and C1s [22] are shown in Table 1. For the purposes of this discussion, SERP-1 provides the most complete set of kinetic data for the reaction of a single serpin with multiple proteinases.

Fig. 1 shows that a plot of $\log k_{inh}$ against $\log K_I$, constructed using the data from Table 1, reveals the apparent existence of a linear free energy relationship (LFER) with slope $\beta = -0.7$. The observation of such a relationship is somewhat surprising: K_I reflects the balance between the rates for the formation and decomposition of EI* which, according to the minimal kinetic mechanism in Scheme 1A, occur through unrelated sequential processes. However, the relationship in Fig. 1 implies that the enzyme–serpin interactions and other factors that are responsible for the variations in k_{inh} between the different serpin–enzyme pairs also govern the magnitude of K_I. In contrast, the wide variations in the relative magnitudes of k_3 and k_4 among the enzymes listed in Table 1, and the lack of any relationship between k_3/k_4 and k_{inh} or K_I, implies that the interactions responsible for discrimination between the enzymes in the transition state for the k_4 step are not related to the interactions leading to variations among the rates of [EI] deacylation in the k_3 step. This conclusion is consistent with the belief that the steps with rate constants k_3 and k_4 represent quite distinct processes: in one case, the enzyme-catalyzed deacylation of [EI] to release cleaved serpin; in the other, a conformational rearrangement involving insertion of the remaining uninserted portion of the RCL into β-sheet A of the serpin structure [20], with concomitant loss of interaction between the enzyme active site and serpin residues on the P1′ (N-terminal) side of the cleavage site within the RCL, a substantial change in the geometry of interaction between the enzyme and residues of the RCL on the C-terminal side of the cleavage site [2], and possibly also a distortion of enzyme active site residues themselves [28].

The LFER shown in Fig. 1 is easily understood, however, if the formation of EI* is truly (if slowly) reversible, as has been shown for some other serpin–proteinase pairs [17–19]. If this

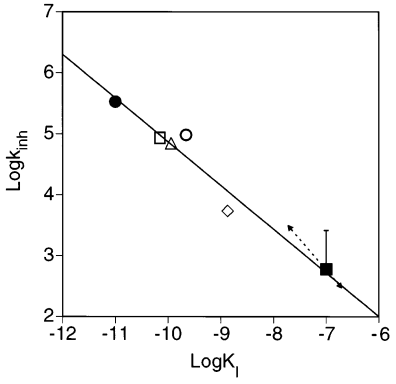


Fig. 1. Linear free energy relationship for the reactions of SERP-1. Linear free energy relationship between $\log k_{inh}$ and $\log K_I$ based on the data from Table 1. The solid line represents the best fit to the data for thrombin (●), tPA (□), uPA (△), plasmin (○), Xa (◇) and C1s (■), and has a slope of $\beta \sim -0.7$. The error bar on the data point for C1s indicates the higher position it would occupy if it were plotted based on the higher value of $k_{app} = 1300\ M^{-1}\ s^{-1}$ reported by Lomas et al. [27]. Data for C1s were estimated based on an assumed SI of 2, to give $k_{inh} = 600\ M^{-1}\ s^{-1}$, and $K_I = 100$ nM [22]; the dashed arrow indicates how the location of the data point for C1s would change for SI values from 1 (bottom right) to 10 (upper left), which span the likely range for this enzyme [22].

is correct, then k_{inh} and K_I comprise describe the rate and equilibrium constants for the same process. As such, it would not be surprising that the interactions that govern the variations in k_{inh} are retained in the final inhibited complex, leading to the observation of an LFER between k_{inh} and K_I. Indeed, the slope of $\beta = -0.7$ would imply that these interactions are approximately two-thirds formed in the transition state for the conformational rearrangement of [EI] relative to their final state in EI* [29,30].

4. Free energy profiles for the serpin–proteinase reaction

The mechanistic significance of the relationship shown in Fig. 1, and of the variations in the other rate constants in Table 1 for the different serpin–enzyme pairs, can be further analyzed in terms of their effects on the shape of free energy profile for the reaction. A free energy profile for the formation and decomposition of EI* can be constructed under two different sets of assumptions, depending on whether the formation of the final inhibited complex (EI*) from [EI] is considered to be irreversible (Scheme 1A) or reversible (Scheme 1B). Under case A (Scheme 1A), the conversion of [EI] to EI* is assumed to be essentially irreversible (i.e. $k_5 \gg k_{-4}$), and therefore the predominant pathway for the decomposition of EI* is through its direct hydrolysis with rate constant k_5. The

Table 1
Summary Of SERP-1 inhibition kinetics

Enzyme	$K_{I(app)}$ (nM)	k_{app} ($M^{-1}\ s^{-1}$)	k_{diss} (s^{-1})	SI	K_I (nM)	k_{inh} ($M^{-1}\ s^{-1}$)
uPA	0.16	5.0×10^4	8×10^{-6}	1.4	0.11	7.0×10^4
tPA	0.14	4.3×10^4	7×10^{-6}	~2	0.07	8.6×10^4
Plasmin	0.44	4.8×10^4	2×10^{-5}	2.0	0.22	9.6×10^4
Thrombin	0.13	2.6×10^4	3×10^{-6}	13	0.01	3.4×10^5
Factor Xa	4.3	1.7×10^3	7×10^{-6}	3.2	1.3	5.4×10^3
C1s	200	3×10^2	6×10^{-5}	n.d.	~100	$\sim6\times10^2$

Kinetic parameters for the reaction of SERP-1 with six inhibited proteinases before and after correction for the effects of partitioning in the branched kinetic mechanism. The values are as reported in Nash et al. [22].

formation of [EI] is also assumed to be irreversible, and thus the partitioning of [EI] through the inhibition and substrate pathways is fast compared to its formation (i.e. k_3, $k_4 \gg k_{-2}$). The relatively low values observed for k_{inh} are taken to indicate that the chemical step (k_2), and not the diffusional encounter of serpin with the proteinase (k_1), is the rate-limiting step in the formation of EI*. These case A assumptions can be represented in the form of a hypothetical free energy profile for the serpin–proteinase reaction, as shown in Fig. 2A. Fig. 2A illustrates how the case A assumptions allow the rate constants k_{inh} and k_{diss} to be associated with specific activation barriers in the free energy profile for reaction. The consequences of the variations in k_{inh} and k_{diss} seen in Table 1 for the shape of the free energy profile can be analyzed by converting the measured differences in rate constants into differences in the free energy of activation for the appropriate step, using the expression $\Delta G^{\ddagger}_{(1)} - \Delta G^{\ddagger}_{(2)} = -RT\ln(k_{(1)}/k_{(2)})$. Application of these case A assumptions to the kinetic data in Table 1 thus results in a slightly different free energy profile for reaction with each enzyme, depending on the values for k_{inh} and k_{diss} that were observed in each case. Fig. 2B shows how the data for reaction with each enzyme influence the shape of the free energy profile, when the kinetic data are interpreted under the case A assumptions that were derived from Scheme 1A.

Under case B (Scheme 1B), the formation of [EI] and its conversion to EI* are considered to be reversible. Evidence exists for several enzyme–serpin systems to suggest that this description is accurate in at least some cases [17–19,31]. Direct hydrolysis of EI* is assumed to be very slow, so that the predominant pathway for the decomposition of EI* is through the reverse of its formation (i.e. $k_5 \ll k_{-4}$). Under these assumptions, for interactions where the stoichiometry of inhibition is relatively low (i.e. where $k_3 < \sim 10k_4$) the formation of [EI] must be fast compared to its partitioning through the inhibition and substrate pathways (i.e. $k_{-2} \gg k_3$, k_4). If this were not true, then the reversion of EI* to [EI] would lead predominantly to the formation of cleaved serpin, and the recovery of active serpin from the complex [17–19,31] would not be possible. Thus, under case B assumptions the rate-limiting step in the formation of EI* is the conversion of [EI] to EI* with rate constant k_4. Adoption of case B assumptions therefore corresponds to the free energy profile shown in Fig. 3A. Application of these assumptions to the kinetic data in Table 1 produces the overlaid free energy profiles shown in Fig. 3B.

The differences in the specificity of SERP-1 for the six inhibited proteinases shown in Table 1 are manifested as variations in the magnitudes of the constants k_{inh}, k_{diss}, K_I and SI. Because we do not know whether case A or case B best describes the reactions of SERP-1 with the proteinases that were tested, application of these two cases leaves us with two distinct and mutually exclusive interpretations of the data. If case A is correct (i.e. $k_{-4} \ll k_5$), then variations in the rate of formation of the final inhibited complex, EI*, arise from differences in the interactions between the enzyme and SERP-1 in the transition state for the formation of [EI] (Fig. 2B). This transition state is presumably that for the chemical step of acylation of the enzyme by SERP-1. The enzymes uPA, tPA, plasmin, and thrombin are therefore inactivated very rapidly because they have more favorable binding interactions

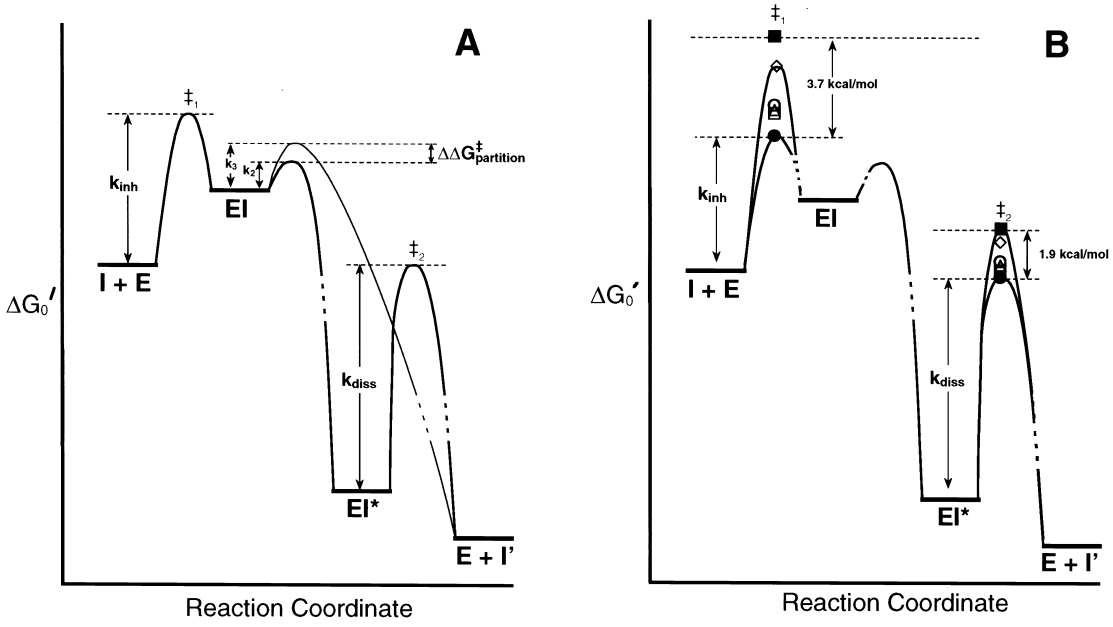


Fig. 2. Free energy profiles for the inhibition of serine proteinases by SERP-1 based on the mechanism shown in Scheme 1A. Broken lines indicate energy barriers for irreversible steps, or barriers that cannot be defined using the kinetic constants in Table 1. A: Free energy profile for the reaction of a proteinase with SERP-1, for the case where the sole pathway for the breakdown of the final inhibited complex is through the direct hydrolysis of EI* to release cleaved serpin, with rate constant k_5 (Scheme 1A). Under this assumption and its corollaries (collectively referred to in the text as the 'case A assumptions'), variations in k_{inh} reflect differences in the activation barrier for the reaction of E+I to form [EI], while variations in k_{diss} reflect differences in the activation barrier for the deacylation of EI* via the pathway with rate constant k_5. The observed variations in K_I reflect the balance between these two independent processes and, consequently, K_I has no intrinsic thermodynamic significance (see text). B: Experimental data from Table 1 for the reactions of SERP-1 with thrombin (●), tPA (□), uPA (△), plasmin (○), Xa (◇) and C1s (■), mapped onto a free energy profile of the form shown in A, as described in the text. For clarity, the k_3 pathway, which is shown in A, has been omitted from B. Free energies were calculated from the data in Table 1 using the relation: $\Delta G^{\ddagger}_{(1)} - \Delta G^{\ddagger}_{(2)} = -RT\ln(k_{(1)}/k_{(2)})$.

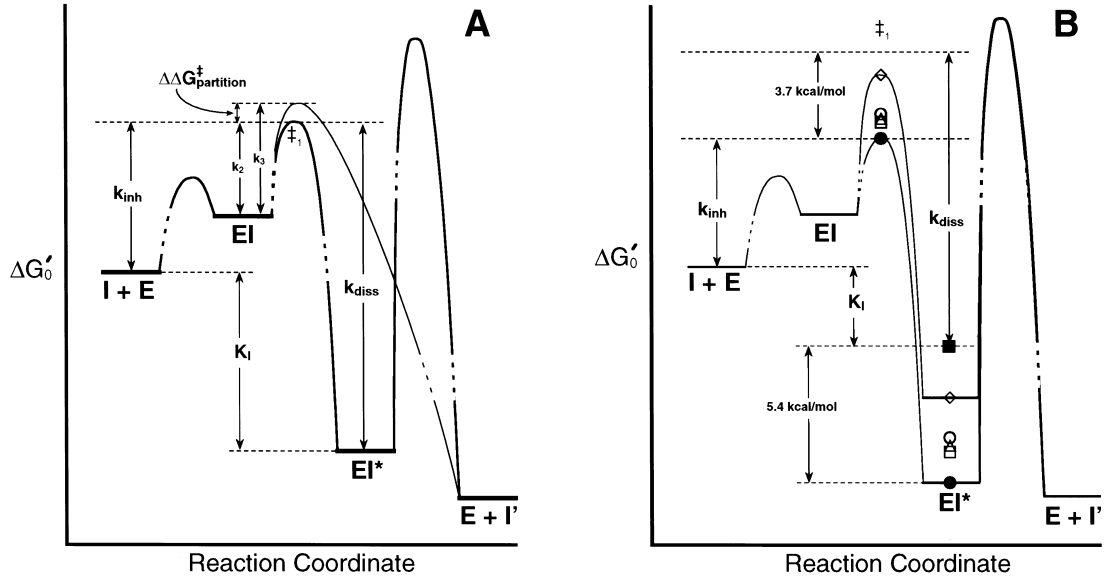


Fig. 3. Free energy profiles for the inhibition of serine proteinases by SERP-1 based on the mechanism in Scheme 1B. Broken lines indicate energy barriers that cannot be defined using the kinetic constants in Table 1. A: Free energy profile for the reaction of a proteinase with SERP-1, for the case where the predominant pathway for the breakdown of the final inhibited complex (under conditions where $[\text{SERP-1}]_{\text{free}} \ll K_I$) is through reversion to [EI] leading to the release of active serpin (i.e. $k_5 \ll k_{-4}$, and $k_3 \ll k_{-2}$; see Scheme 1B). Under these assumptions (collectively referred to in the text as the 'case B assumptions'), variations in k_{inh} represent variations in the stability of the transition state, relative to the E+I ground state, for the conversion of [EI] to EI*; and variations in K_I reflect variations in the stability of the final inhibited complex, also with respect to the E+I ground state (see text). This set of assumptions is thus consistent with the experimental observation that the variations in k_{inh} and in K_I are quantitatively related through the LFER shown in Fig. 1. B: Experimental data from Table 1 for the reactions of SERP-1 with thrombin (●), tPA (□), uPA (△), plasmin (○), Xa (◇) and C1s (■), mapped onto a free energy profile of the form shown in A, as described in the text. For clarity, the k_3 pathway, which is shown in A, has been omitted from B. Free energies were calculated from the data in Table 1 using the relations: $\Delta G^{\ddagger}_{(1)} - \Delta G^{\ddagger}_{(2)} = -RT\ln(k_{(1)}/k_{(2)})$, and $\Delta G^{\text{Eq}}_{(1)} - \Delta G^{\text{Eq}}_{(2)} = -RT\ln(K_{(1)}/K_{(2)})$.

with SERP-1 in the transition state for this step than do the more slowly inhibited enzymes factor Xa and C1s. Under these assumptions, the variations in k_{diss} reflect differences in the activation barrier for the deacylation of EI* via the pathway with rate constant k_5, resulting from some combination of variations in ground state and transition state stabilities. Thus, under the case A assumptions, the variations in K_I reflect the balance between these two independent effects and, as a consequence, have no intrinsic thermodynamic significance and cannot be interpreted to reflect changes in the free energy of EI*.

Under the case B assumptions, variations in the rate of the formation of EI* arise from differences in the interactions between the enzymes and SERP-1 in the transition state for the conformational change in which [EI] converts to EI* (Fig. 3B). Variations in K_I have a more direct interpretation than was the instance in case A. K_I can now be seen to directly reflect differences in the thermodynamic stability of EI*, with stabilities of these final inhibited complexes varying over some 5.4 kcal/mol in the order thrombin > tPA ~ plasmin ~ uPA > Xa > C1s (Fig. 3B).

Under both case A and case B assumptions, the variations in SI reflect variations in the partitioning of [EI] between inhibition and substrate pathways. For example, partitioning of [EI] through the substrate pathway to release cleaved serpin is much more favorable, relative to the formation of EI*, for uPA than it is for thrombin.

5. Conclusions and future directions

Examination of Figs. 2B and 3B shows that the LFER seen in Fig. 1 is fully consistent with the case B assumptions, derived from Scheme 1B, but is harder to reconcile with case A (Scheme 1A). Fig. 3B shows that if case B is correct, variations in k_{inh} and K_I result from changes in the free energies of the transition state and product state, relative to the E+I ground state, for the same microscopic step; that is for the conversion of [EI] to EI*. The magnitude of the Brønsted coefficient of $\beta = -0.7$, obtained from the slope of the plot in Fig. 1, indicates that the variations in transition state stability result from differences in enzyme–serpin interactions that are roughly two-thirds developed in the transition state relative to their strengths in the final inhibited complex, EI*. In contrast, Fig. 2B shows that under case A assumptions the variations in K_I result from differences in enzyme–serpin interactions that occur in two unrelated steps. As such, under case A assumptions there is little reason to expect a correlation between k_{inh} and K_I of the quality that is seen in Fig. 1. Although, in principle, it is possible to distinguish experimentally whether the step with rate constant k_{-4} or the step with rate constant k_5 represents the predominant pathways for the breakdown of EI*, and such measurements have been made in a few cases [19], in practice such experimental tests are quite difficult. The observation of the linear correlation shown in Fig. 1 provides support for the case B assumptions that $k_{\text{diss}} = k_{-4}$, and therefore that $K_I = k_{-4}/k_{\text{inh}}$, since no correlation between k_{inh} and K_I would be expected if $K_I = k_5/k_{\text{inh}}$, as is required in case A. Nevertheless, the observation of the correlation shown in Fig. 1 does not provide conclusive proof of a thermodynamic link between k_{inh} and K_I, and we therefore regard the nature of the dissociation pathway in the reaction to remain an open question.

Regardless of which interpretation on this point is correct, our results show unequivocally that, although the inhibitory specificity of SERP-1 is substantially influenced by variations in the interactions between enzyme and serpin in the transition state of the rate-limiting step in the formation of EI*, specific binding interactions in ground states or transition states that occur after the rate-limiting step for inhibition may also play a significant role. The strength of the interaction between enzyme and serpin in these later complexes, and also the partitioning between inhibition and substrate pathways, may therefore contribute to the inhibitory specificity of serpins that act at concentrations close to their IC_{50} for interaction with a given proteinase, or that are not present in significant excess over their proteinase targets, as has been discussed more fully elsewhere [22]. These conditions may pertain to the anti-inflammatory role of SERP-1, as well as to situations such as the inhibition of intracellular caspases by crmA or other serpin inhibitors of apoptosis, where the serpin may be acting at relatively low concentrations.

Acknowledgements: This work was supported by the Medical Research Council of Canada, National Cancer Institute (Canada) and Biogen, Inc. P.N. is a Medical Research Council (Canada) post-doctoral fellow, G.M. is a Medical Research Council of Canada Senior Scientist. We wish to thank Peter Gettins, Christopher Borysenko, and the late Stuart Stone for thought-provoking discussions.

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