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Bovine trypsinogen activation

A thermodynamic study

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The *N*-α-L-isoleucyl-L-valine (Ile-Val) activating dipeptide, sequentially homologous to the Ile 16-Val 17 N-terminus of bovine β-trypsin, displays an activating effect on equilibria involved in the binding of strong ligands (i.e., *n*-butylamine and the porcine pancreatic secretory trypsin inhibitor (Kazal-type inhibitor, type I; PSTI)) to bovine trypsinogen. This property has been investigated between pH 3.0 and 9.0 ($I = 0.1$ M) at 21.0 °C. The thermodynamics for the interaction of strong ligands with bovine β-trypsin has also been studied under the same experimental conditions. The equilibria involved in the binding of the Ile-Val activating dipeptide and/or inhibitors to bovine β-trypsin and its zymogen are described according to linkage relationships, wherefore interaction(s) between different functional and structural domains of the (pro)enzyme (i.e., the so-called Ile-Val pocket and the primary and/or secondary recognition subsite(s)), possibly involved in the bovine trypsinogen-to-β-trypsin activation pathway, are considered.

1. Introduction

Inhibitor association to serine proteinases and to their zymogens, which represents a valuable model for macromolecular recognition, has been shown to be a complex event, being characterized by stepwise conformational changes of the protein(s) after the initial interaction. Subsequently, the effects of inhibitor and activator binding have been taken as a valuable model for the zymogen-to-active proteinase process [1–8]. In particular, the bovine trypsinogen-to-β-trypsin activation event has been thoroughly investigated from structural [3–5,8–13], dynamic [14–16] and functional [2,7,10,17–24] viewpoints, leading to clear-cut evidence for a coupling mechanism between the so-called activation domain and the (pro)enzyme active-site region. The structural rearrangements responsible for the functional effect can be properly induced (albeit to a partial extent) in bovine trypsinogen not only by the binding to the Ile-Val pocket of activating dipeptides mimicking the N-terminal residues of bovine β-trypsin (as resulting from limited proteolytic cleavage, i.e., the initial step of the activation process), but also by the association of strong ligands to the reactivity center [2,7,10,17–24].

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The present analysis has been focussed on (i) the thermodynamics of strong ligand (i.e., *n*-butylamine and the porcine pancreatic secretory trypsin inhibitor (Kazal-type inhibitor, type I; PSTI)) binding to bovine (β-)trypsin(ogen), which may be taken as a molecular model for serine (pro)enzyme inhibition [2,7,8,25]; and (ii) the relationship of such reactions with the general phenomenon of proenzyme activation, which is mimicked by the binding of the *N*-α-L-isoleucyl-L-valine (Ile-Val) activating dipeptide (sequentially related to the Ile 16-Val 17 N-terminus of bovine β-trypsin) and strong ligands (i.e., substrates and inhibitors). The conformational transitions associated with this event are especially important for proteinases, since the control of the activation process is crucial to the optimal functioning of the bovine (β-)trypsin(ogen) system. In this paper, such a coupling effect between the Ile-Val- and/or inhibitor-linked functional changes occurring in the bovine (β-)trypsin(ogen) system has been quantitatively analyzed as a function of pH, using linkage relationships [26].

2. Materials and methods

Bovine β-trypsin, treated with diphenylcarbamyl chloride in order to abolish chymotryptic activity, was purified from commercial enzyme preparations (Sigma, St. Louis, MO, U.S.A.) as previously reported [27]. Bovine trypsinogen was obtained from Merck (Darmstadt, F.R.G.); zymogen preparations were found to be virtually free of trypsin, chymotrypsin, elastase and kallikrein activities [20]. PSTI (type I) was prepared and purified as described previously [28]. The bovine basic pancreatic trypsin inhibitor (Kunitz-type inhibitor; BPTI) was kindly provided by Lepetit S.p.A. (Milano, Italy) and purified according to previously reported methods [20,29,30].

The homogeneity of bovine β-trypsin, bovine trypsinogen, PSTI and BPTI was checked by polyacrylamide gel electrophoresis with SDS, in the presence and absence of β-mercaptoethanol, according to the procedure of Weber et al. [31]. The preparations used contained less than 3% of nonenzymatic and/or noninhibitory protein contaminants.

Bovine β-trypsin and bovine trypsinogen concentrations were determined (i) by titration with *p*-nitrophenyl *p*-guanidinobenzoate [32–34], and (ii) spectrophotometrically, the $E^{1\%}_{1\,cm}$ values being 15.6 at 278 nm for bovine β-trypsin [35], and 13.9 at 280 nm for bovine trypsinogen [36]. The values of the bovine β-trypsin and bovine trypsinogen concentrations obtained by the two methods agree very well (> 95%). PSTI and BPTI concentrations were determined spectrophotometrically at 280 nm, using $E^{1\%}_{1\,cm}$ values of 5.18 [37], and 8.3 [30], respectively.

n-Butylamine, *p*-nitrophenyl *p*-guanidinobenzoate and *N*-α-carbobenzoxy-L-lysine *p*-nitrophenyl ester (ZLysONp) were purchased from Sigma. The Ile-Val activating dipeptide was synthesized as detailed previously [20]. All other products were obtained from Merck. All chemicals were of analytical grade and used without further purification.

The thermodynamics for *n*-butylamine and PSTI binding to bovine β-trypsin and bovine trypsinogen, the latter in the presence and absence of Ile-Val, has been evaluated on the basis of the inhibitory effect on the bovine β-trypsin- and bovine trypsinogen-catalyzed hydrolysis of ZLysONp. The (pro)enzyme-catalyzed cleavage of ZLysONp was followed spectrophotometrically at 360 nm, the $\Delta\epsilon_{360}$ values changing from 4.5 mM^{-1} cm^{-1} at pH ≤ 5.5 to 10.2 mM^{-1} cm^{-1} at pH ≥ 8.5 with a pK value of 7.1 [7,20,38].

Values of the association equilibrium constant for binding of *n*-butylamine and PSTI to the (pro)enzyme (K_{obs}), in the presence and/or absence of Ile-Val, were determined from the dependence of the molar fraction of the inhibited (pro)enzyme on the free concentration of *n*-butylamine or PSTI, taking into account the simple competition of both inhibitors with ZLysONp for bovine β-trypsin and bovine trypsinogen [7]. An average error value of ±8% was ascribed as the standard deviation to K_{obs} values [7,19,21].

Under all experimental conditions, the following ranges for free reagent concentrations were employed: [bovine β-trypsin], 1.0×10^{-10}–1.0×10^{-8} M; [bovine trypsinogen], 1.0×10^{-6}–$1.0 \times$

10^{-4} M; [*n*-butylamine], 1.0×10^{-5}–1.0 M; [PSTI], 3.2×10^{-8}–1.7×10^{-2} M; [Ile-Val], 8.0×10^{-6}–2.0 M [22,23]; [ZLysONp], 1.0×10^{-5}–1.0×10^{-2} M.

In order to prevent any catalytic effect of bovine β-trypsin, possibly arising from autoactivation of the proenzyme during the reaction time, 0.1 mol BPTI/mol bovine trypsinogen was added to each reaction mixture according to Bode [17].

Calcium ($CaCl_2$, 2.0×10^{-2} M) was always present in the system; the calcium concentration has an indirect effect on the equilibria of *n*-butylamine and PSTI binding to bovine trypsinogen, modulating the thermodynamics of Ile-Val association to the zymogen and to its adducts with strong ligands (i.e., substrates and inhibitors), the maximal effect being at calcium concentrations exceeding 1.0×10^{-3} M [17]. On the other hand, the calcium concentration does not affect strong ligand binding to bovine β-trypsin [20].

All data were obtained in formate buffer (pH 3.0–4.2), acetate buffer (pH 3.5–6.0), Bistris-HCl buffer (pH 5.3–7.8) and Tris-HCl buffer (pH 6.8–9.0) ($I = 0.1$ M) at 21.0° C. According to Antonini et al. [19,20] and Menegatti et al. [24], control experiments with different buffers overlapping in pH showed no specific ion effects.

The spectrophotometric measurements were carried out using Varian Cary 219 and Jasco J-510 double-beam spectrophotometers.

The atomic coordinates used for computer graphics modeling of the (pro)enzyme adducts discussed have been recovered from the Brookhaven Protein Data Bank distribution tape [39].

Data analysis was carried out on a Digital PDP 11/23 computer employing an iterative nonlinear least-squares fitting procedure according to a Marquardt algorithm.

3. Results and discussion

Under all experimental conditions, *n*-butylamine and PSTI binding to (Ile-Val:)bovine(β-)trypsin(ogen) conforms to simple equilibria. Moreover, as expected for simple systems, the values of the observed association equilibrium constant for *n*-butylamine and PSTI binding to (Ile-Val:) bovine (β-)trypsin(ogen) (i.e., K_{obs} values) are independent of the (pro)enzyme concentration. Next, for all the systems considered, the values of the thermodynamic parameters obtained are in excellent agreement with those reported in the literature [7,20,21,23].

Over the entire pH range explored (pH 3.0–9.0), the affinity of *n*-butylamine and PSTI for bovine trypsinogen (as expressed in terms of K_{obs}) increases with the Ile-Val concentration (see figs 1 and 2). According to linkage relationships [26], this finding indicates that, in the zymogen, the Ile-Val binding pocket is functionally linked to the subsite(s) at which *n*-butylamine and PSTI associate [7,20,21,23] in such a way that the affinity for one ligand is increased as a result of binding of the other. The simplest model [7,21,26] for such behavior (figs 1 and 2) leads to the following expression (eq. 1)

$$\log K_{obs} = \log C + \log \frac{(1 + K_{LIG}[\text{Ile-Val}])}{(1 + K_{UNL}[\text{Ile-Val}])} \quad (1)$$

where C denotes a constant that corresponds to the K_{obs} value obtained in the absence of the Ile-Val activating dipeptide, [Ile-Val] the free Ile-Val activating dipeptide concentration, and K_{UNL} and K_{LIG} the association equilibrium constants for Ile-Val binding to the inhibitor-free bovine trypsinogen and to its adducts with *n*-butylamine and PSTI, respectively. Eq. 1 has been used to generate the curves shown in figs 1 and 2; the agreement with experimental data is fully satisfactory (see figs 1 and 2).

The activating effect of Ile-Val (as well as of other dipeptides sequentially related to the Ile 16-Val 17 N-terminus of bovine β-trypsin [7]) on PSTI binding to bovine trypsinogen is greater than that observed for proenzyme:*n*-butylamine adduct formation (see figs 1–4), indicating that, apart from the interaction(s) occurring between the S_1 subsite (the unique *n*-butylamine-binding area) and the Ile-Val pocket, additional interactions between the secondary subsites and Ile-Val cleft come into play for PSTI binding. Differential functional states of bovine trypsinogen find structural support [3,5,8–13,17] in the proenzyme conformational transitions (induced by activating di-

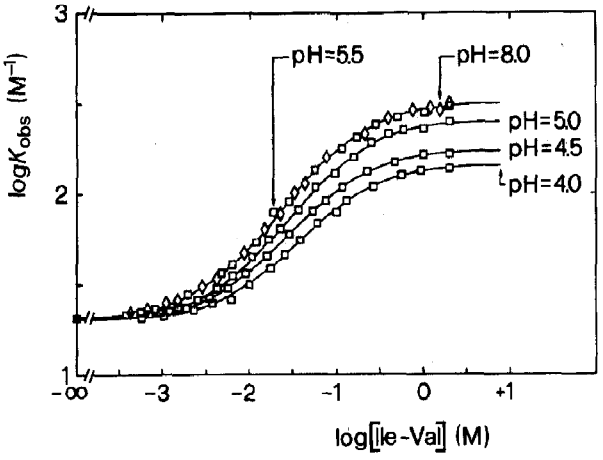

Fig. 1. Effect of Ile-Val concentration (log[Ile-Val]) on the observed association equilibrium constant (log K_{obs}) for *n*-butylamine binding to bovine trypsinogen as a function of pH ($I = 0.1$ M; 2.0×10^{-2} M $CaCl_2$) at 21.0 °C. The filled symbol on the left ordinate refers to the pH-independent K_{obs} value obtained in the absence of Ile-Val (corresponding to *C*). The concentration of Ile-Val is that of the free activating dipeptide [7]. The continuous lines are the best-fit curves calculated via an iterative nonlinear least-squares procedure according to eq. 1 with the choice of K_{UNL} and K_{LIG} values given in table 1. An average error of ±12% was determined for K_{UNL} and K_{LIG} values as the standard deviation. For further details, see text.

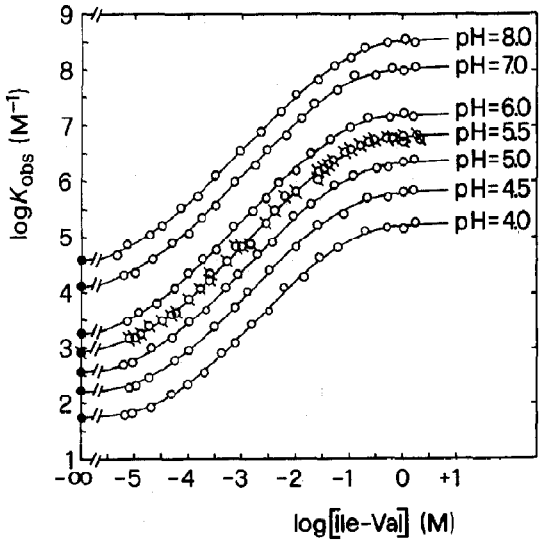

Fig. 2. Effect of Ile-Val concentration (log[Ile-Val]) on the observed association equilibrium constant (log K_{obs}) for PSTI binding to bovine trypsinogen as a function of pH ($I = 0.1$ M; 2.0×10^{-2} M $CaCl_2$) at 21.0 °C. Data denoted by diagonal lines (\) were taken from Ascenzi et al. [7,21]. The filled symbols on the ordinate refer to the pH-dependent K_{obs} values obtained in the absence of Ile-Val (corresponding to *C*). The concentration of Ile-Val is that of the free dipeptide [7]. The continuous lines are the best-fit curves calculated using an iterative nonlinear least-squares procedure according to eq. 1 with the choice of K_{UNL} and K_{LIG} values given in table 1. An average error of ±12% was determined for K_{UNL} and K_{LIG} values as the standard deviation. For further details, see text.

peptide binding) which extends beyond the S_1 subsite to areas involved in secondary interactions with the PSTI reactive loop (see fig. 5).

Even though different activating dipeptides display large differences in affinity for the proenzyme(:strong ligand) adducts, the activating effect is independent of the dipeptide sequence, indicating the same overall result whenever the heterotropic site is occupied [7].

Under all experimental conditions, the lower affinity of the Ile-Val activating dipeptide for free bovine trypsinogen as compared to that for the proenzyme adducts with *n*-butylamine and PSTI (table 1) reflects the less structured state of the Ile-Val binding pocket in the free zymogen relative to that in the proenzyme : inhibitor adducts, and the lower degree of rigidity of the Ile-Val binding cleft induced in trypsinogen by *n*-butylamine than that brought about by PSTI. Next, the different pH effects on Ile-Val binding to free bovine trypsinogen (K_{UNL} being pH-independent) and to bovine trypsinogen : inhibitor adducts (K_{LIG} decreasing on lowering the pH) (table 1) reflect the difference in the geometry of the Asp 194 residue (located at the bottom of the Ile-Val binding pocket and modulating the association of the activating dipeptide) in the proenzyme and in its adducts with strong ligands [3,5,7,8,10–13].

Throughout the entire pH range explored (pH 3.0–9.0) the K_{obs} values for *n*-butylamine binding to free trypsinogen are pH independent; in contrast, the affinity of *n*-butylamine for the Ile-Val : bovine trypsinogen adduct and bovine β-trypsin decreases with pH, following the acidic pK'_H shift, upon ligand binding, of a single ionizing group (see figs 1 and 3). Similarly, the K_{obs} values for PSTI binding to bovine trypsinogen, in both the absence and presence of Ile-Val, and to

bovine β-trypsin decrease with pH, following different titration profiles. In particular, (i) between pH 9.0 and 5.0, the pH dependence of K_{obs} follows the acidic pK''_H shift of a single ionizing group, for PSTI binding to free bovine trypsinogen and its adduct with Ile-Val, as well as to bovine β-trypsin (see figs 2 and 4); (ii) below pH 5.5, inhibitor association to free bovine trypsinogen can be described by the acidic pK'_H shift of two ionizing groups upon PSTI binding where, within the same pH range, the formation of adducts between PSTI and Ile-Val : proenzyme, as well as bovine β-trypsin, is modulated by the acidic pK'_H shift of three ionizing residues (see figs 2 and 4). According to linkage relationships [26], these findings signify that, in (Ile-Val)bovine (β-)trypsin(ogen), the proton-binding residues are functionally linked to the subsite(s) at which *n*-butylamine and PSTI associate [2,8,19,20,24] in such a way that the affinity of one ligand is decreased as a result of binding of the other. The

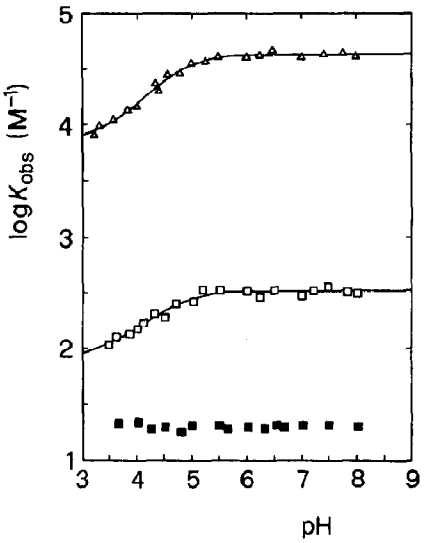

Fig. 3. Effect of pH on the observed association equilibrium constant (log K_{obs}) for *n*-butylamine binding to free bovine trypsinogen (■), Ile-Val : bovine trypsinogen adduct (i.e., in the presence of saturating concentrations of the activating dipeptide, 2.0 M Ile-Val (□)) and bovine β-trypsin (△) ($I = 0.1$ M; 2.0×10^{-2} M $CaCl_2$) at 21.0 °C. Values of K_{obs} for *n*-butylamine binding to free bovine trypsinogen are pH-independent with an average value of 22.0 ± 2.0 M^{-1}. The continuous lines are the best-fit curves calculated via an iterative nonlinear least-squares procedure according to eq. 2, assuming the acidic pK'_H shift, upon *n*-butylamine binding to the Ile-Val : bovine trypsinogen adduct and bovine β-trypsin, of a single ionizing group ($K'_F = 4.0\times10^{-5}$ M; $K'_B = 1.6\times10^{-4}$ M; $n = 1$; $K''_F = K''_B$) between pH 3.0 and 5.5. An average error of $\pm12\%$ was determined for K'_F and K'_B values as the standard deviation. For further details, see text.

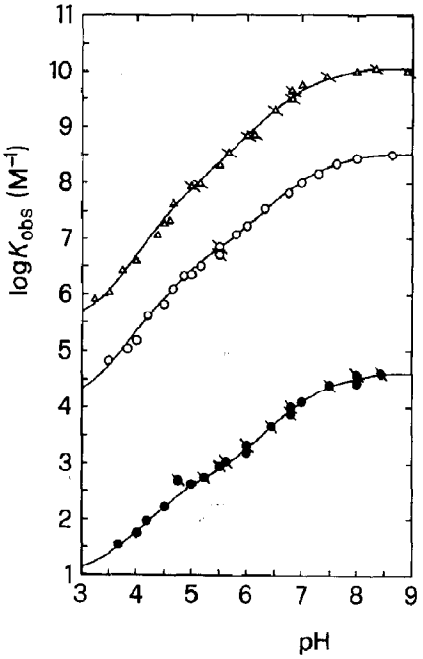

Fig. 4. Effect of pH on the observed association equilibrium constant (log K_{obs}) for PSTI binding to free bovine trypsinogen (●), Ile-Val : bovine trypsinogen adduct (i.e., in the presence of saturating concentrations of the activating dipeptide, 2.0 M Ile-Val (○)) and bovine β-trypsin (△) ($I = 0.1$ M; 2.0×10^{-2} M $CaCl_2$) at 21.0 °C. Data denoted by diagonal lines (\) were taken from Antonini et al. [19] and Ascenzi et al. [7,21]. The continuous lines are the best-fit curves calculated via an iterative nonlinear least-squares procedure according to eq. 2, assuming the acidic pK_H shift, upon PSTI binding to free bovine trypsinogen, of two equivalent ionizing groups ($K'_F = 3.1\times10^{-5}$ M; $K'_B = 1.9\times10^{-4}$ M; $n = 2$) between pH 3.0 and 5.5, and of a single ionizing group ($K''_F = 4.2\times10^{-8}$ M; $K''_B = 4.2\times10^{-6}$ M) between pH 5.0 and 9.0; and, upon association of inhibitor to the Ile-Val : bovine trypsinogen adduct and bovine β-trypsin, of three equivalent ionizing groups ($K'_F = 7.1\times10^{-5}$ M; $K'_B = 1.9\times10^{-4}$ M; $n = 3$) between pH 3.0 and 5.5, and of a single ionizing group ($K''_F = 5.0\times10^{-8}$ M; $K''_B = 6.3\times10^{-6}$ M) between pH 5.0 and 9.0. An average error of $\pm12\%$ was determined for K'_F, K'_B, K''_F and K''_B values as the standard deviation. For further details, see text.

Table 1

Association equilibrium constants for binding of Ile-Val activating dipeptide to free bovine trypsinogen (K_{UNL}) and to its adducts with *n*-butylamine and PSTI (K_{LIG}) at 21.0 °C

System	pH	K_{UNL} ($\times 10^{-1}$) (M^{-1}) [a]	K_{LIG} ($\times 10^{-2}$) (M^{-1}) [a]
Bovine trypsinogen/*n*-butylamine	4.0	1.0	7.0×10^{-1}
	4.5	1.2	1.0
	5.0	1.0	1.3
	5.5	1.0	1.7
	6.0	1.1	1.7
	7.0	1.0	1.7
	8.0	1.1	1.7
Bovine trypsinogen/PSTI	4.0	1.0	3.2×10^{2}
	4.5	1.1	4.3×10^{2}
	5.0	1.0	6.6×10^{2}
	5.5	1.0	8.3×10^{2}
	6.0	1.1	1.1×10^{3}
	7.0	1.1	9.2×10^{2}
	8.0	1.0	1.1×10^{3}

[a] Thermodynamic parameters were determined from analysis of the data given in figs 1 and 2 according to eq. 1. An average error of ±12% was evaluated for K_{UNL} and K_{LIG} values as the standard deviation.

simplest model [2,19,20,24,26] for such a behavior (figs 1–4) leads to the following expression:

$$\log K_{obs} = \log E - \log \frac{(1 + K'_F[H^+])^n (1 + K''_F[H^+])}{(1 + K'_B[H^+])^n (1 + K''_B[H^+])} - \log \frac{K'^n_B K''_B}{K'^n_F K''_F} \quad (2)$$

where E denotes a constant that corresponds to the alkaline asymptote of K_{obs}, $[H^+]$ the proton concentration, K'_F, K''_F, K'_B and K''_B the proton dissociation equilibrium constants for the inhibitor-free (K_F) and inhibitor-bound (K_B) (Ile-Val:)bovine (β-)trypsin(ogen), and n the number of amino acid residues modulating inhibitor association below pH 5.5. Eq. 2 has been used to generate the curves shown in figs 2 and 4; the agreement with experimental data is fully satisfactory (see figs 2 and 4).

The observed pH effects find support in several structural considerations [3,8–11,13,19,20]; thus, the pH dependence of K_{obs} for *n*-butylamine

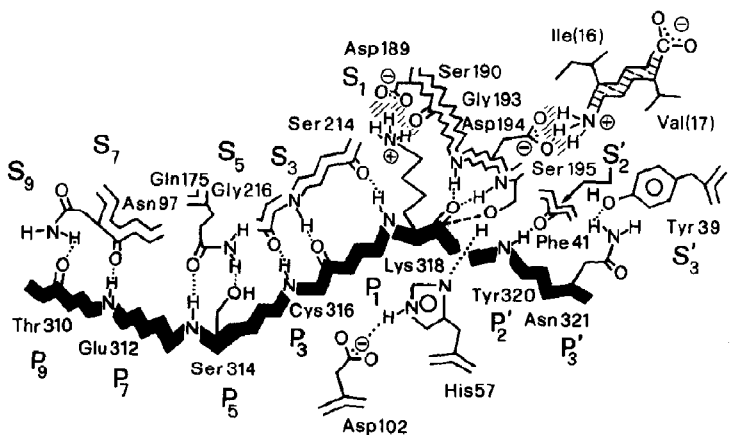

Fig. 5. Schematic drawing of the main- and side-chain polar interactions between bovine (Ile-Val:)(β-)trypsin(ogen) (double main-chain connections) and PSTI (solid main-chain connections) occurring at the (pro)enzyme/inhibitor contact area. The Ile (16)-Val (17) binding mode to its specific pocket at the (pro)enzyme activation domain is shown in the upper right-hand corner. The amino acid residues forming the (pro)enzyme catalytic triad (i.e., His 57, Asp 102 and Ser 195) are also shown. Amino acid residues are identified by their three-letter codes and sequence numbers. The numbering of the (pro)enzyme refers to that of homologous bovine chymotrypsinogens A and B [41]. For the sake of clarity, the PSTI residue numbers have been increased by 300 [13]. The (pro)enzyme subsites have been identified according to Schechter and Berger [42]. Atomic coordinates of the (Ile-Val:)bovine (β-)trypsin(ogen)(:PSTI) adduct were taken from Bode and Schwager [9], Bode et al. [11] and Bolognesi et al. [8,13]. For further details, see text.

binding to the Ile-Val : bovine trypsinogen adduct and to bovine β-trypsin may be consistent with the acidic pK'_H shift, upon ligand binding, of residue Asp 189. In fact, the negatively charged side chain of Asp 189 interacts with the positively charged amidino group of *n*-butylamine, the ε-amino group of Lys 18 in native PSTI and of Lys 15 in native BPTI, and the δ-guanidino group of Arg 15 in semisynthetic BPTI. On the other hand, the pH independence of bovine trypsinogen : *n*-butylamine adduct formation reflects the absence of any pK'_H shift of Asp 189, upon ligand binding, suggesting this residue to be solvent-inaccessible in both the free zymogen and in its adduct with *n*-butylamine. By analogy, this structural aspect should be responsible for the different pH profile of PSTI binding to free bovine trypsinogen with respect to the Ile-Val : zymogen adduct and to bovine β-trypsin, indicating that the acid-base equilibrium of Asp 189 does not modulate the interaction of PSTI with free bovine trypsinogen whereas it does play a role in the case of the Ile-Val-activated proenzyme and of bovine β-trypsin. One of the residues that is likely to affect PSTI binding to (Ile-Val:)bovine (β-)trypsin(ogen) might be Asp 102, which is involved in the catalytic triad of serine (pro)enzymes. Such a linkage has been suggested for the bovine β-trypsin : BPTI complex on the basis of the pH dependence of the ^{1}H-NMR signal assigned to the proton hydrogen-bonded between Asp 102 and His 57ND1; in fact, this signal disappears when the pH is lowered to 3.5 [40]. As reported previously for pH effects on BPTI and PSTI binding to serine (pro)enzymes [2,19,24], no plausible assignment is possible for the third acidic ligand-linked ionizable group, which could reflect pH-dependent conformational changes occurring beyond the (pro)enzyme : inhibitor contact area. Next, lowering pH from 9.0 to 5.0, the decrease in the affinity of PSTI for the proenzyme, in the absence and presence of Ile-Val, and for bovine β-trypsin is probably related to the acidic pK''_H shift, upon inhibitor binding, of the His 57 side chain, involved in the catalytic triad of serine (pro)enzymes, as reflected in the strengthening of the Ser 195 OG-His 57 NE2 hydrogen bond, which is observed in all the adducts of bovine (β-)trypsin(ogen) with macromolecular inhibitors but which is very weak or absent in the free (pro)enzyme. Such a form of control is ineffective, between pH 9.0 and 5.0, on *n*-butylamine binding to (Ile-Val:)bovine(β-)trypsin(ogen) (figs 1 and 3); indeed, at variance with macromolecular inhibitor association, *n*-butylamine interacts only with the (Ile-Val:)(pro)enzyme S_1 subsite without involving His 57 [3,4,8] (fig. 5).

It should be mentioned that over the whole pH range explored, the Ile-Val : bovine trypsinogen adduct shows K_{obs} values for *n*-butylamine and PSTI binding that are much more favorable than those of the free zymogen, although in no case were they comparable to those reported for bovine β-trypsin (figs 1–4). Evidence in support of the limited activation effect being induced in the bovine trypsinogen(:inhibitor) adduct upon Ile-Val binding is provided by crystallographic observations [3,5,8–13] which show that activating dipeptides give rise to less complete rigidification of the zymogen's activation domain loops than that induced by the endogenous N-terminal dipeptide of β-trypsin. The conformations assumed by trypsinogen upon binding of Ile-Val and/or inhibitor may indeed correspond to different activation states involved in the bovine trypsinogen-to-β-trypsin activation transition, which may therefore be considered as a multistep event rather than an all-or-nothing process [1,5,7,14,19–23,25].

On the whole, these results may be taken as representative of the molecular and functional model for the activation transitions occurring in the serine (pro)enzymes belonging to the chymotrypsin superfamily in view of the proteinase homology and the equivalence between the Ile-Val activating dipeptide and the Ile 16-Val 17 N-terminal of the active enzymes [1–3,5,7,8,17,41].

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