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Inhibition of Bacterial DD-Peptidases (Penicillin-Binding Proteins) in Membranes and in Vivo by Peptidoglycan-Mimetic Boronic Acids

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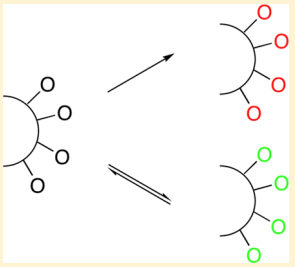
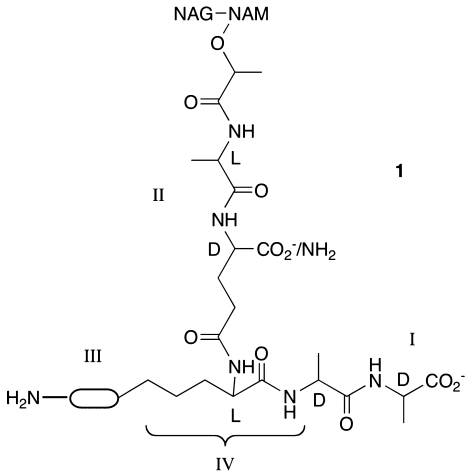
ABSTRACT: The DD-peptidases or penicillin-binding proteins (PBPs) catalyze the final steps of bacterial peptidoglycan biosynthesis and are inhibited by the β-lactam antibiotics. There is at present a question of whether the active site structure and activity of these enzymes is the same in the solubilized (truncated) DD-peptidase constructs employed in crystallographic and kinetics studies as in membrane-bound holoenzymes. Recent experiments with peptidoglycan-mimetic boronic acids have suggested that these transition state analogue-generating inhibitors may be able to induce reactive conformations of these enzymes and thus inhibit strongly. We have now, therefore, measured the dissociation constants of peptidoglycan-mimetic boronic acids from *Escherichia coli* and *Bacillus subtilis* PBPs in membrane preparations and, in the former case, in vivo, by means of competition experiments with the fluorescent penicillin Bocillin Fl. The experiments showed that the boronic acids bound measurably (K_i < 1 mM) to the low-molecular mass PBPs but not to the high-molecular mass enzymes, both in membrane preparations and in whole cells. In two cases, *E. coli* PBP2 and PBP5, the dissociation constants obtained were very similar to those obtained with the pure enzymes in homogeneous solution. The boronic acids, therefore, are unable to induce tightly binding conformations of these enzymes in vivo. There is no evidence from these experiments that DD-peptidase inhibitors are more or less effective in vivo than in homogeneous solution.



The DD-peptidases [penicillin-binding proteins (PBPs)] catalyze the final transpeptidase, carboxypeptidase, and endopeptidase reactions of bacterial cell wall biosynthesis. They are the targets of β-lactam antibiotics, which inhibit them by active site acylation.[1−6] Bacterial resistance to β-lactams is fierce and includes the very efficient hydrolysis of β-lactams by the serine and metallo-β-lactamases, the former group of which is structurally and mechanistically related to the DD-peptidases.[7,8] In view of this long-established and intense resistance to β-lactams, DD-peptidase inhibitors with structures other than those based on the β-lactam ring, and thus not necessarily affected by β-lactamases, have long been of interest. It is striking, however, that no natural products comparable effectiveness to β-lactams as DD-peptidase inhibitors have yet been discovered, despite extensive screening of natural sources.[9,10]

In recent years, screening of synthetic chemical libraries has revealed micromolar noncovalent inhibitors of specific DD-peptidases as potential leads for the development of non-β-lactam inhibitors.[11−14] None of them, however, has yet been translated into an antibiotic. Boronic acids have recently been identified as covalent (but reversible) inhibitors.[15−18] Certain boronic acids also possess bacteriocidal activity against Gram-positive organisms.[18] Boronic acids,[19−21] like phosphonates[22,23] and sulfonates,[24] generate tetrahedral, covalent inhibitory adducts at serine hydrolase active sites, which mimic transition states of acyl transfer reactions.[25,26]

Because many very effective enzyme inhibitors are substrate or transition state analogues, it seems rational to approach the design of DD-peptidase inhibitors by this strategy. Unfortunately, the substrate specificity of DD-peptidases, in general, is not well-understood.[27] One would anticipate that these enzymes would exhibit noticeable affinity for some element of peptidoglycan structure (**1**). Results to date show that such affinity depends very much on the class of DD-peptidase



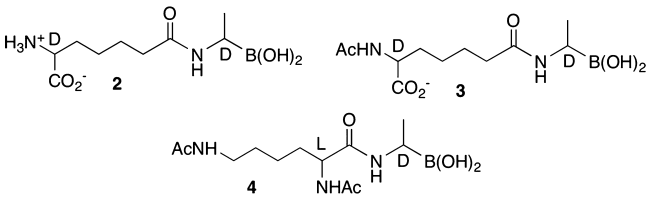
involved. On the basis of amino acid sequence comparisons, DD-peptidases are divided into two major classes, high molecular mass (HMM) and low molecular mass (LMM), where the dividing line is ∼50 kDa.[3] These groups are subdivided, the former into subgroups A and B and the latter into

Received: February 1, 2012
Revised: March 9, 2012
Published: March 23, 2012

A−C. Although general activities in vivo, transpeptidase, carboxypeptidase, and endopeptidase, have been ascribed to these subclasses,[4−6,28] in many cases the element of peptidoglycan that the enzyme specifically recognizes is unknown. Experiments designed to test the affinity of DD-peptidases for small, but distinctive, elements of peptidoglycan, represented as segments I, II, and III in **1**, and combinations of these, have revealed that LMMB and LMMC enzymes have strong affinity for the combination of segments I and III[29,30] and, in fact, contain a binding site specific to segment III.[31−33] These results derive from experiments with peptidoglycan-mimetic peptides, depsipeptides,[35,36] β-lactams,[37,38] and boronates.[20] Essentially all evidence to date[29,30,34,35] has not revealed any such specific binding on LMMA and HMM DD-peptidases.[27,29,38,39] It was observed, however, that in certain LMMA and LMMC enzymes, boronate inhibitors showed some degree of peptidoglycan specificity even in cases where none was observed in peptide hydrolysis.[40] It was proposed that specificity to structural elements of peptidoglycan might be induced by stable transition state analogues, both in vitro and, more importantly, in vivo.

A question related to the proposition of the last sentence is that of the conformation of the DD-peptidases in solution. Are the solubilized constructs of the LMMA and HMM enzymes that are used for solution kinetics studies and crystal structures present in (fully) active conformations in solution? There is structural evidence that this might not always be so,[27,41−46] which may relate to the observed limited activity of many of these enzymes, even against peptidoglycan-mimetic peptides.

To investigate these issues, we have studied the inhibition of the DD-peptidases of *Escherichia coli* and *Bacillus subtilis*, in isolated membranes and in vivo, by the peptidoglycan (segment III)-mimetic boronates **2**−**4**. Inhibition constants (K_i) of the



boronates were obtained from competitive kinetics employing the fluorescent β-lactam Bocillin Fl[47,48] as a reference irreversible inhibitor. Appropriate quantitative treatment of the data from these experiments and the derived inhibition constants are discussed.

MATERIALS AND METHODS

Bacterial Strains and Reagents. *E. coli* strain MM 294 (wild type) was kindly provided by D. B. Oliver (Department of Molecular Biology and Biochemistry, Wesleyan University). *B. subtilis* strain A 198 (wild type) was obtained from the Bacillus Genetic Stock Center (The Ohio State University, Columbus, OH). Bocillin Fl was purchased from Invitrogen, and benzylpenicillin was purchased from Aldrich. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS−PAGE) precast gels (Mini-PROTEAN TGX) were purchased from Bio-Rad, and bands were visualized with a Typhoon Trio variable mode imager [General Electric; green laser (532 nm), AlexaFluor 532 filter]. The boronic acid inhibitors were synthesized as previously described.[20,40] Purified enzymes, *E. coli* PBP5 and PBP2, were generously supplied by R. A. Nicholas (University of North Carolina, Chapel Hill, NC) and H. Adachi (University of Tokyo, Tokyo, Japan), respectively.

Growth of Bacterial Cells and Membrane Isolation. *E. coli* cells were grown and membranes isolated as described in ref 39. Membranes were resuspended (10 mg of protein/mL) in ice-cold 20 mM phosphate buffer (pH 7.2) containing 140 mM NaCl. *B. subtilis* cells were grown and membranes isolated as described in ref 49. Membranes were resuspended (15 mg of protein/mL) in ice-cold phosphate buffer, as described above. Protein concentrations were determined by the Bradford method.[50] Estimates of the antimicrobial sensitivity of *E. coli* to the boronic acids (MIC values) were obtained as previously described.[39]

Rate Measurements in Vivo. *E. coli* cells were grown in Difco LB broth medium for 6 h at 37 °C with vigorous aeration at 220 rpm. Cells were centrifuged at 4000 rpm for 10 min and washed twice with ice-cold 20 mM phosphate buffer (pH 7.2) containing 140 mM NaCl. Cells from 25 mL of culture were resuspended in 0.5 mL of ice-cold phosphate buffer and used immediately. For measurements of the rate constants for reaction of the PBPs with Bocillin Fl, 10 μL aliquots of the *E. coli* cell suspension were used in a 50 μL reaction mixture. Appropriate volumes of a Bocillin Fl stock solution (1 mM), prepared in water, were added to the samples to yield the required concentrations (10−200 μM), the mixtures incubated for 2 min at room temperature, and then the reactions quenched with benzylpenicillin (5 μL, 12 mg/mL). Subsequently, the samples were centrifuged (4000 rpm) to collect the cells; phosphate buffer (15 μL) was added to each pellet, and the samples were subjected to three freeze−thaw cycles. Aliquots of a 1% Triton X-100 solution (10 μL) and Laemmli buffer[51] (3×, 15 μL) were then added, and the samples were boiled for 10 min, centrifuged (4000 rpm), and subjected to SDS−PAGE. For K_i measurements, different concentrations of boronate inhibitors (0−1500 μM) were included in the reaction mixtures, which were incubated for 30 min at room temperature prior to addition of Bocillin Fl (5 μL, 20 μM).

Rate Measurements in Membranes. For measurement of the rate constants for reaction of membrane-bound PBPs with Bocillin Fl, appropriate volumes of a stock solution (1 mM) of the latter were added to aliquots (2.0 μL) of the membrane preparation in phosphate buffer; final Bocillin Fl concentrations ranged from 10 to 200 μM in a total volume of 8.0 μL. Samples were incubated for 2 min at room temperature and reactions quenched with benzylpenicillin (1.0 μL, 12 mg/mL). Laemmli buffer[51] (4× stock solution, 3 μL) was then added, and the samples were boiled for 3 min, centrifuged at 4000 rpm, and subjected to SDS−PAGE. Inhibition constants were determined with the same protocol except that a 40 min incubation of the membranes with the boronate inhibitor (0−1500 μM) was included prior to addition of Bocillin Fl. The same procedures were used to determine the rate constants of purified enzymes (*E. coli* PBP2 and PBP5). These were added as aliquots (2 μL) of 0.5 μM stock solutions.

***B. subtilis* Sporulation Rates.** *B. subtilis* cells were grown in Schaeffer's sporulation medium (SSM)[52] at 37 °C and aerated by being shaken at 200−220 rpm in the presence and absence of boronic acid **2** (0.1 mM). The appearance of spores was judged visually by reference to cells grown normally in LB medium.

Data Analysis. Equation 1, describing the concentration of E-I_1 (and hence F) as a function of time in the presence of the fast reversible inhibitor I_2, was derived from Scheme 3.

dx.doi.org/10.1021/bi300148v | *Biochemistry* 2012, 51, 2804−2811

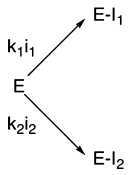
In Scheme 3, I_1 and I_2 represent Bocillin Fl and boronate, respectively. The enzyme reacts irreversibly with Bocillin Fl to form E-I_1 with a second-order rate constant k_1 and was assumed to form fast reversible complexes EI_2 with the boronates, the latter with dissociation constants K_2. F and F_{max} represent the intensities of the PBP bands on the PAGE gel when the initial concentration of I_2 is i_{20} and when $i_{20} = 0$ and $t = \infty$ (when all of the enzyme would be labeled with Bocillin Fl), respectively. With the reaction protocols employed, t was a constant and the independent variable in eq 1 was i_{20} (concentrations represented in lowercase). To obtain k_1, eq 1 with an i_{20} of 0 was employed. The gel intensity data were fit to these equations by a nonlinear least-squares procedure to first obtain values for k_1 and then K_2.

RESULTS AND DISCUSSION

Inhibition constants for interaction of small molecule inhibitors with membrane-bound DD-peptidases have been determined for many years from competition experiments with β-lactams bearing either radioactive, fluorescent, or biotin labels.[53] Reaction of these enzymes with a nonlabeled inhibitor is monitored by the competitive reaction of the free, unreacted enzyme with the labeled compound. These experiments can be conducted either competitively or consecutively. Often, IC_{50} values are reported, based on the concentration of the inhibitor required to produce 50% of the total signal. The meaning of IC_{50}, however, depends on the experimental protocol. Further, and often, the time dependence of the reactions concerned has not been sufficiently taken into consideration, leading to IC_{50} values that are incorrectly interpreted or not interpreted at all, except comparatively. To use these methods correctly, we first briefly outline the common experiments and their interpretation. In all of these reactions and protocols, the inhibition is assumed to be competitive and reactions are run under pseudo-first-order conditions, i.e., $i_0 \gg e_0$.

An Irreversible Inhibitor, Usually Another β-Lactam. *Competitive Experiment (Scheme 1).* In this experiment, the concentration of E-I_1, the labeled enzyme, is monitored. Often, for convenience and high throughput, e.g., in a multiwell plate screening experiment, the experiment is quenched at a chosen time t. Under these conditions

Scheme 1[a]



[a] I_1 is the labeled reference β-lactam, and I_2 is the unlabeled analogue.

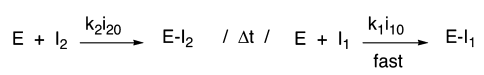
$$\frac{F(\text{measured intensity})}{F_{max}(\text{intensity when } i_{20} = 0)} = \frac{(k_1 i_{10})/k_2}{(k_1 i_{10})/k_2 + i_{20}}$$

where concentrations are represented by the lowercase i and the the zero subscript refers to initial conditions.

From this experiment, therefore, $IC_{50} = (k_1 i_{10})/k_2$, and the measured IC_{50} is therefore a function of k_1, i_{10}, and k_2 and is independent of t. For a given reference inhibitor and initial concentration, IC_{50} will depend on only k_2 (faster reacting I_2 will give lower IC_{50} values) and relative IC_{50} values will reflect relative reactivities. Note that it is assumed in Scheme 1 that the reactions are strictly second-order. This is generally true for β-lactams at low (micromolar) concentrations, but if not, and an initial noncovalent binding step is significant, the situation is more complicated;[54] this also applies to the schemes below.

Consecutive Experiment (Scheme 2). In this case, the reaction between E and I_2 is allowed to proceed for some time,

Scheme 2



Δt, and then quenched by addition of I_1 under conditions such that unreacted E is rapidly trapped by I_1. Under this protocol

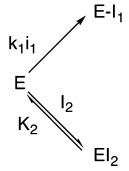
$$\frac{F}{F_{max}} = e^{-k_2 i_{20} \Delta t}$$

where F_{max} is the measured fluorescence intensity when $\Delta t = 0$ and $IC_{50} = 0.693/(k_2 \Delta t)$.

Here IC_{50} is Δt-dependent but, with a constant quenching time, is inversely proportional to k_2, as in the Competitive Experiment.

A Reversible Inhibitor (Scheme 3). In this case, fast reversible binding of I_2 is assumed and thus the experiment is conducted competitively. Under these conditions

Scheme 3



$$\frac{F}{F_{max}} = 1 - \exp[-(k_1 i_{10} t)/(1 + i_{20}/K_2)] \quad (1)$$

where F_{max} is the fluorescence intensity when $i_{20} = 0$ and $t = \infty$ and $IC_{50} = K_2[(k_1 i_{10} t/0.693) - 1]$.

Thus, IC_{50} is directly proportional to K_2, but its absolute value is a function of i_{10} and t (this is still true if F_{max} is defined as the fluorescence intensity when $i_{20} = 0$ at time t, but in this case, the proportionality constant is more complicated). It is noticeable that the response curve (eq 1) is exponential, although in the literature it is often fit to a hyperbolic function, as, for example, recently, in ref 55.

One would expect the boronates **2**–**4** to generally behave as fast reversible inhibitors (Scheme 3), at least in cases with micromolar dissociation constants. With tighter binding, slower association and, more particularly, dissociation might be an issue. Dissociation of **2** from *B. subtilis* PBP4a, for example, is "slow", where the dissociation is only complete after a few minutes.[40] Certain aryl boronates dissociate at similar slow rates from the *Actinomadura* R39 DD-peptidase.[21] Such cases, under the assay conditions of this paper, would generally not yield accurate values of K_2 and IC_{50}. Rigorous treatment of these cases is, however, possible.

E. coli and *B. subtilis* PBPs, isolated in membranes, were incubated with boronates **2**–**4**, the association probed by means of Bocillin Fl, and analyzed by means of Scheme 3 and eq 1, as described above. Typical PAGE gel assay data are

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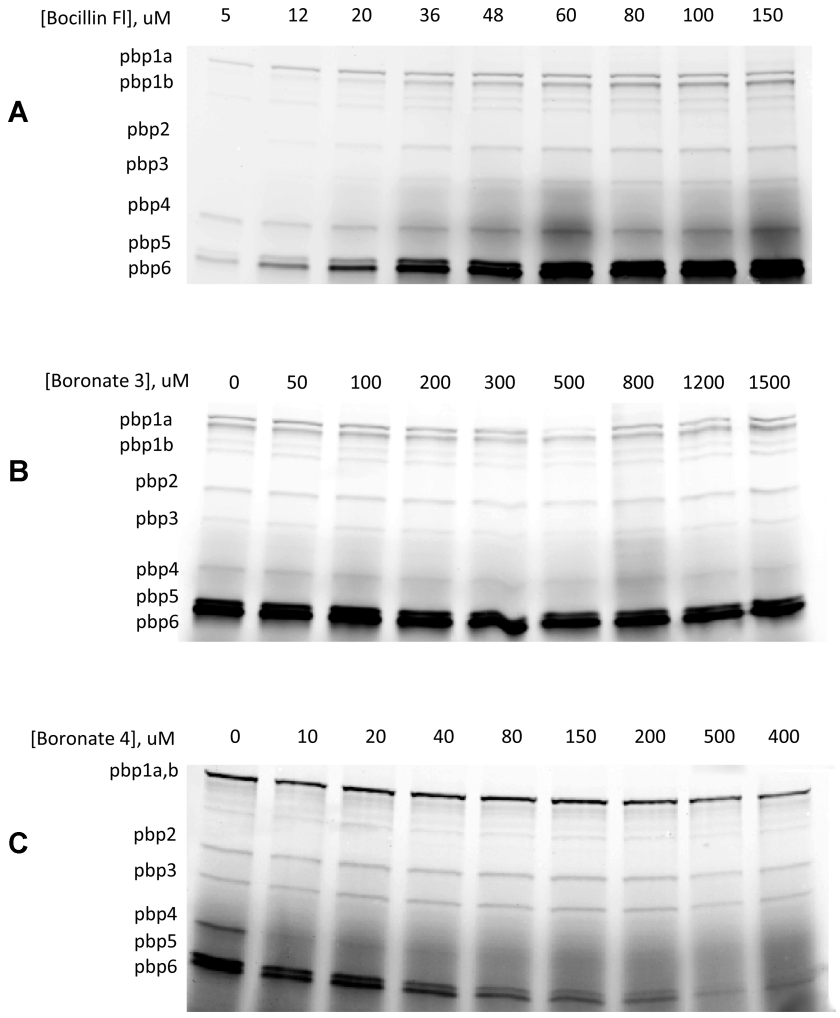

Figure 1. (A) Extent of fluorescent labeling of *E. coli* PBPs by Bocillin Fl as a function of the concentration of the latter. (B) Extent of labeling of *E. coli* PBPs by Bocillin Fl (20 μM) in the presence of various concentrations of boronic acid **3**. (C) Same as panel B with 20 μM Bocillin Fl and boronic acid **4**. Other details of these experiments are given in Materials and Methods.

shown in Figure 1 and fits of the data to eq 1 in Figure 2A. For each PBP, the rate constant for reaction with Bocillin Fl was first determined, as also described above, with the resulting k_1 values for *E. coli* listed in Table 1. As expected, in cases where association with the boronic acid occurs, the fluorescence intensity of the particular PBP decreases with inhibitor concentration (Figure 2B). Analysis of these data by means of eq 1 led to the K_2 values of Table 2. These values could be reproduced between separate experiments within 20%.

Bocillin Fl, a penicillin, seemed strikingly reactive with PBP4; benzylpenicillin itself appears to react with all of the *E. coli* PBPs with similar rates,[56] as also observed in Table 1 with Bocillin Fl, except for PBP4.

We will now address boronic acid inhibition of the *E. coli* LMM enzymes because we have comparable data from measurements made in a homogeneous aqueous solution (for PBP5 in particular, using a solubilized construct lacking the membrane-associating N-terminal peptide[40]). Boronates **2** and **3**, where the structure of **2** is a direct mimic of the stem peptide terminal amine (segment III in **1**), bind weakly to PBP5 and PBP6. PBP5 and PBP6 are structurally very similar,[40,57] so their similarity of boronate binding is not surprising; we note also that **4** binds to PBP5 and PBP6 with similar strength. There appears to be no specificity for segment III, however, on comparison of the constants for **2** and **3**. This observation agrees with the results for PBP5 in solution,[40] also given in Table 2.

The striking feature of the data for PBP5 and PBP6 is the greater effectiveness of **4** as an inhibitor versus **2** or **3**, both in free solution and bound to the membrane. Thus, although the boronate moiety is unable to induce specificity toward the free stem peptide structure, it does appear to do so, to some degree, when a neutral N-terminus is present. This result does mimic peptide substrate specificity, because although **5** is a poor substrate of PBP5, it is at least 1 order of magnitude better (k_{cat}/K_m) than the closer peptidoglycan-mimetic **6**.[40] Larger neutral N-acyl groups do not seem to promote substrate specificity, either in PBP5[40,58] or in the closely similar *Neisseria gonorrheae* PBP4.[40,59] Larger *N*-acyl groups do, however, allow a boronate to induce a more functional active site conformation in PBP5.[19,40] Thus, although no strong affinity for PBP5 and PBP6 by either peptidoglycan-mimetic peptides or boronates has been demonstrated, either in solution or in *E. coli* membranes, the data from our experiments and previous ones with peptides[40] do indicate that the most likely role for these enzymes is as DD-carboxypeptidases acting on polymeric peptidoglycan.

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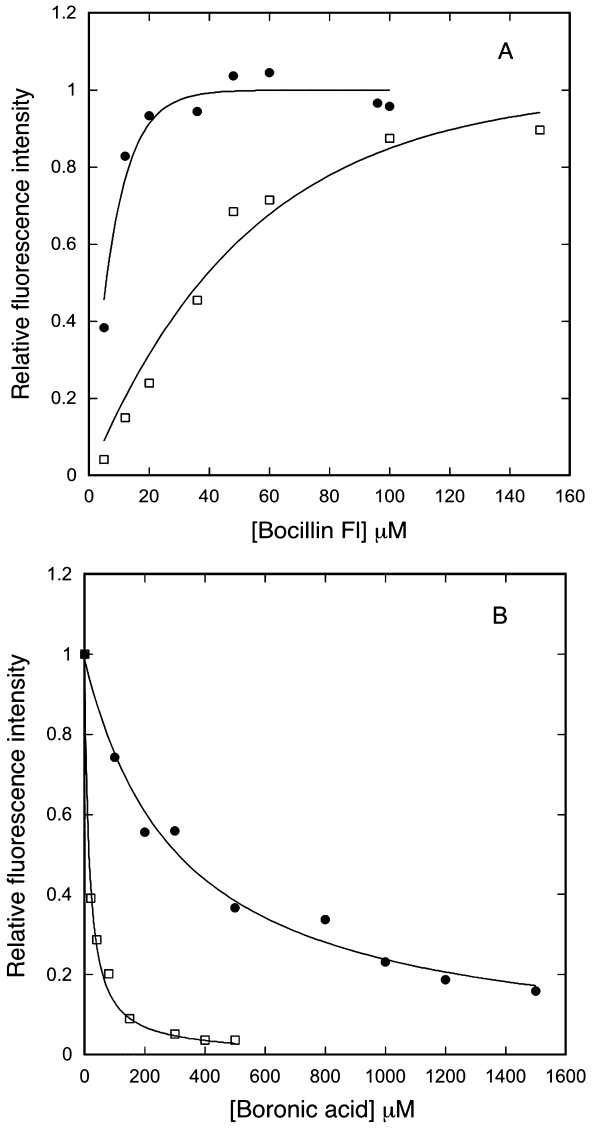


Figure 2. (A) Extent of fluorescent labeling of *E. coli* PBP1a (●) and PBP6 (□) by Bocillin Fl as a function of the concentration of the latter. The points are experimental, derived from gels such as shown in Figure 1, and the curves derive from the fitting of the data to eq 1. In each case, the ordinate is normalized to the observed final intensity. (B) Extent of Bocillin Fl (20 μM) labeling of *E. coli* PBP5 (●) as a function of boronic acid **2** concentration and of *E. coli* PBP6 (□) in the presence of boronic acid **4**. In each case, the ordinate is normalized to the observed initial intensity. Other details of these experiments are given in Materials and Methods.

Table 1. Rate Constants for Reaction of Bocillin F1 with *E. coli* PBPs in Membranes

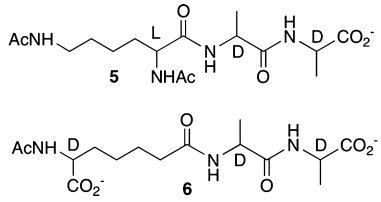
PBP	k_1 (s^{-1} M^{-1})
1a	500 ± 10
1b	95 ± 2
2	165 ± 16
3	121 ± 3
4	(7.9 ± 1.5) × 10^3
5	100 ± 25
6	160 ± 25

It has been established that LMMC DD-peptidases contain a specific binding site for a free N-terminus (segment III) in

Table 2. Inhibition of *E. coli* PBPs by Boronic Acids

	K_2 (μM)		
PBP	**2**	**3**	**4**
1a/b	>1000	>1000	>1000
2	>1000	>1000	>1000
		>1000[a]	
3	>1000	>1000	>1000
4	0.010 ± 0.001	1.0 ± 0.3	0.25 ± 0.04
5	250 ± 20	540 ± 140	5.6 ± 0.6
	330 ± 30[a]	650 ± 110[a]	14.5 ± 1.1[a]
	>1000[b]		86 ± 9[b]
6	750 ± 150	>1000	13.7 ± 0.6

[a]Purified enzyme in solution. [b]In vivo; rate constant k_1 for Bocillin F1 was 70 ± 16 s^{-1} M^{-1}.



peptidoglycan-mimetic substrates.[32,33] This is observed in catalytic activity toward specific peptides,[29,36,40] rapid acylation by specific β-lactams,[38] and strong binding of specific boronates.[40] Evidence of this site is also seen in Table 2, where boronate **2** shows a significantly greater affinity for PBP4 in membranes than does **3** or **4**. The crystal structure and amino acid sequence of PBP4 show clear evidence of a D-aminopimelyl binding site.[41,60]

Finally, the membrane-bound HMM PBP1a, -1b, -2, and -3 show no measurable affinity for boronates **2** and **3** (Table 1). Measurement of the affinity of solubilized PBP2 for **2** in dilute aqueous solution produced the same result. This lack of affinity mimics the absence of, or at best minimal, activity of these enzymes against peptidoglycan-mimetic D-alanyl-D-alanine-terminated peptides.[2,29,38,61,62]

Results for *B. subtilis* PBPs in membranes were very similar to those for *E. coli*. No inhibition of the HMM enzymes (PBP1, -2a, -2b, -2c, -3, and -4[63]) by **2** and **3**, both at 1.0 mM, was observed (not shown). The LMMA enzyme PBP5, which is very similar to *E. coli* PBP5,[2] was inhibited by boronate **2** (K_2 = 69 ± 3 μM) but not by **3** (at 1.0 mM). This enzyme therefore shows greater specificity toward the stem peptide structure than does *E. coli* PBP5. Although PBP4a is expressed in *B. subtilis* during late vegetative stages,[64] deletion of the gene expressing it does not appear to affect sporulation.[65] We have now observed that growth of *B. subtilis* in the presence of **2**, a powerful inhibitor of PBP4a,[40] does not seem to affect sporulation time either. Another possible role for PBP4a that has been suggested is in biofilm formation.[66]

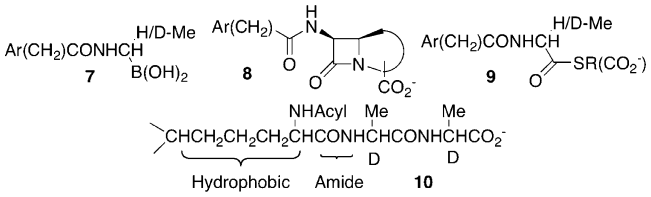
In vivo measurements of the inhibitory power of boronates **2**–**4** against *E. coli* PBPs in intact cells were also made, with results very similar to those in the membranes. The HMM enzymes were not affected. PBP5 was inhibited by **4** (K_2 = 86 ± 9 μM) but not by **2** (at 1.0 mM). The considerably higher value of the K_2 of **4** in vivo versus that found with the enzyme in isolated membranes (14.5 μM) may represent a difference between the active site conformation in vivo and that in the membrane preparations but more likely reflects an access issue, probably caused by the outer cellular membrane. Boronic acid **2**,

dx.doi.org/10.1021/bi300148v | *Biochemistry* 2012, 51, 2804–2811

however, as a low-molecular weight zwitterion, would not be expected to have difficulty with the porins.[67,68] Bocillin Fl itself certainly did not seem to have significant diffusion problems: in free solution the rate constant for its reaction with *E. coli* PBP5 was found to be 116 ± 8 s^{-1} M^{-1}, while in membranes and in vivo the corresponding values were 100 ± 25 and 70 ± 16 s^{-1} M^{-1}, respectively. In another experiment, after incubation of *E. coli* with **2**, cells were removed by centrifugation and the concentration of **2** in the supernatant was assayed by inhibition of *B. subtilis* PBP4a.[40] No decrease in the concentration of **2** was observed, however, and thus, **2** is not depleted by metabolic processes (**2** is an amino acid of course), a phenomenon that could also lead to an apparently lower affinity.

CONCLUSIONS

The peptidoglycan-mimetic boronates **2** and **3** appear to be no more effective as inhibitors of *E. coli* and *B. subtilis* PBPs in membranes and in vivo than in dilute aqueous solution. The environment of PBPs in membranes and in vivo does not produce active site conformational changes that allow **2** and/or **3** to bind tightly to them. It seems unlikely, therefore, that small peptidoglycan-mimetic peptides[40] would be efficient substrates in membranes or in vivo. In striking contrast, boronates and β-lactams of structures **7**[17,18] and **8**, respectively, are potent inhibitors of PBPs; both of these are transition state analogue-generating species. Thioldepsipeptides of structure **9** are often substrates of these enzymes, although usually poor to moderate in absolute terms.[69−71] These molecules are apparently all able to engage the hydrophobic and amide binding sites (**10**; **1**, segment IV), common to all PBPs,[31] and use it to directly access a protein conformation that also tightly binds the transition states of natural substrate turnover or their analogues.



The interaction of these enzymes with peptide substrates is more complicated. To induce acyl transfer chemistry in amides, it seems that the PBPs use either the stem peptide segment III N-terminus (LMMB and LMMC enzymes) or more distant, currently unknown, triggers (HMM, LMMA PBPs). Employment of the latter mechanism may have evolved as resistance to small molecule inhibitors and/or antibiotics. Its existence does, however, make rational antibiotic design against HMM PBP targets quite challenging.

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Funding

This research was supported by National Institutes of Health Grant AI-17986 (R.F.P.).

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

HMM, high molecular mass; LMM, low molecular mass; PBP, penicillin-binding protein.

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