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Lysine N^{ζ}-Decarboxylation in the BlaR1 Protein from *Staphylococcus aureus* at the Root of Its Function As an Antibiotic Sensor

Jooyoung Cha and Shahriar Mobashery*

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556

Received January 22, 2007; E-mail: mobashery@nd.edu

It is predicted that there are approximately 2700 unique folds for proteins in nature.[1] Considering that in the vast majority of organisms the number of proteins exceeds the number of folds, the same protein folds will by necessity perform diverse functions (e.g., approximately 20000−25000 protein-coding genes may exist in human cells,[2] which might translate over 50000 proteins). An impressive example is the structural templates seen for the class D β-lactamases and the antibiotic sensor domain of the BlaR1 protein (Figure 1). The former is an antibiotic resistance enzyme, with the ability to turn the antibiotic over, whereas the latter is a signal sensor/transducer protein in certain Gram-positive bacteria. Both proteins are acylated by β-lactam antibiotics at a serine residue, but only the class D β-lactamase is able to hydrolytically deacylate the acyl-enzyme species to achieve turnover. Once acylation takes place in the BlaR1 protein, the species enjoys longevity, well beyond the doubling time of the bacteria. Therefore, when the acylated species is formed once, it survives the duration of one bacterial generation. On the other hand, class D β-lactamases turn over β-lactam antibiotics many times, such that the resistant bacterium grows unencumbered by the drug. The surface domain of BlaR1 from *Staphylococcus aureus* and the class D OXA-10 β-lactamase from *Pseudomonas aeruginosa* share 26% identity and 49% similarity in their amino acids, producing backbone structures that are virtually identical (Figure 1). A remarkable similarity exists in their respective active sites as well.

An appreciation of the underlying reasons for this divergence of function between these two proteins is emerging only now, with its cornerstone in the presence of a critical N-carboxylated lysine within the active sites.[3−6] This N-carboxylated lysine activates the active site serine for acylation by abstraction of the hydroxyl proton. It passes the proton to the lactam nitrogen, as the acyl-enzyme intermediate forms. As depicted below, the N-carboxylated lysine (as discerned from the X-ray structure for the β-lactamase) makes a bifurcated hydrogen bond to the serine hydroxyl (Scheme 1). Ab initio calculations on this system revealed that the transfer of proton via the carbamate oxygen (route B) results in a stable intermediate. However, the passage of the proton through the carbamate nitrogen results in a barrierless decarboxylation of the N-carboxylated lysine (route A).[3,7] Both these events have been observed. The class D β-lactamase facilitates many substrate turnover events, indicating that it relies primarily on route B.[4] On the other hand, the process is arrested at the acyl-enzyme stage for the BlaR1 protein with the attendant loss of carbon dioxide. This observation is documented now by the X-ray structures for the acyl-enzyme species, by NMR experiments,[3,8] and by stopped-flow FTIR experiments,[6] arguing for predominance of route A for the BlaR1 protein. The consequence of this divergence is of course profound, as it shapes the physiological functions of the two types of proteins entirely—one as an enzyme and the other as a receptor protein.

Edified by this knowledge, we explored if the BlaR1 protein exhibited any β-lactamase activity. To document the potential residual activity, we resorted to the measurement of the so-called

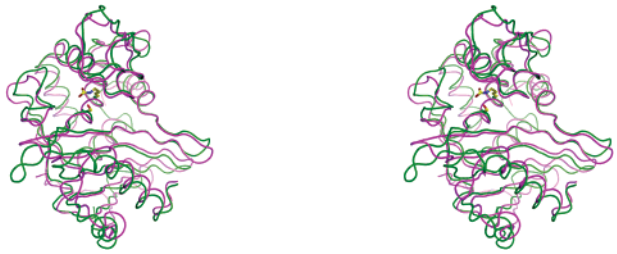

Figure 1. Stereoview of the superimposed peptide backbones from X-ray data of the class D OXA-10 β-lactamase from *Pseudomonas aeruginosa* (PDB code 1K57, green) and the sensor domain of the BlaR1 protein from *Staphylococcus aureus* (1xkz, magenta). Active site serine-lysine pair in the OXA-10 enzymes is shown centrally in capped stick. The lysine residue is shown N-carboxylated. Atoms O, C, and N are in red, yellow, and blue for OXA10, respectively.

partition ratio for a series of β-lactam antibiotics with the sensor domain of BlaR1. In this analysis, the antibiotic is treated as an irreversible inhibitor for the protein. The mathematical basis for the analysis and the analysis itself are described elsewhere.[9,10] The partition ratio is the ratio of k_{cat}/k_{inact}, an indication of the efficiency of the irreversible protein modification. As indicated in Table 1, approximately 1−6 antibiotic molecules were needed to fully modify the protein. This ratio is too small for us to be able to characterize the turnover by steady-state measurements (the k_{cat} event), but it indicates the existence of a residual turnover of β-lactam antibiotics with the surface domain of the BlaR1 protein.

Having documented that residual turnover of antibiotic is seen with the BlaR1 protein, the observation indicates that a few turnover events (route B, Scheme 1) take place before entry into route A with its attendant loss of carbon dioxide. We wondered what would happen if the N-decarboxylation of lysine in the BlaR1 protein were not to take place. Would that be sufficient to convert the structural template of BlaR1 from a receptor to an antibiotic resistance enzyme? To explore this possibility, we developed a method similar to that pioneered by Kirsch in the preparation of γ-thialysine as a surrogate for lysine.[11] We generated a cysteine at position 392 of the BlaR1 protein by mutagenesis (Lys392Cys mutant). Because the site of mutagenesis in protein is sheltered, the protein was denatured to show that 1.0 equiv of thiol could be titrated by 5,5′-dithiobis(2-nitrobenzoic acid)(DTNB). The protein was denatured in degassed 20 mM Tris-HCl, 10 mM EDTA, pH 7.5, supplemented with urea (8 M), and then incubated with 4-iodobutanoate at room temperature in an airtight container. Because 4-iodobutanoate is prone to lactonization, aliquots of the reagent were added every 2 h for a duration of 30 h, at which time DTNB titration revealed the absence of the cysteine thiol. The modified protein exhibited a single protein band by isoelectric focusing. The protein was refolded by a protein-folding column (ProFoldin, Westborough, MA). The proper column for refolding (among several) was chosen on the basis of the comparison of the reaction of nitrocefin (a chromogenic cephalosporin) with the wild-type BlaR1 and that for the refolded wild-type BlaR1.

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Scheme 1

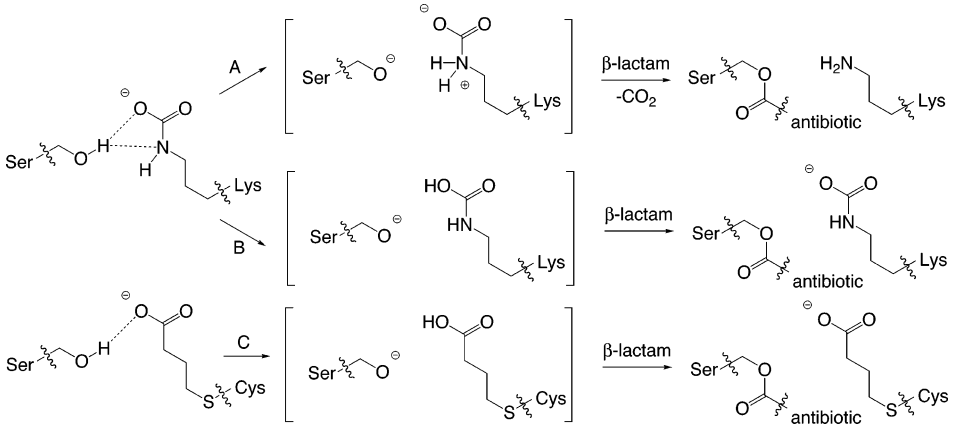

Table 1. Partition Ratios and Steady-State Kinetic Data for the Wild-Type BlaR1 and the *S*-(4-Butanoate)-Cysteine Variant[a]

	partition ratio				
substrate	wild type	modified variant	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($M^{-1}s^{-1}$)
ceftazidime	5.8 ± 0.3	1340 ± 57	2.3 ± 0.2	6 ± 1	(4.0 ± 0.7) × 10^5
cephalosporin C	5.3 ± 0.3	451 ± 30	0.3 ± 0.0	12 ± 2	(2.5 ± 0.4) × 10^4
cephalothin	3.0 ± 0.2	428 ± 50	1.0 ± 0.0	30 ± 3	(3.3 ± 0.3) × 10^4
cefepime	3.1 ± 0.3	1704 ± 90	2.0 ± 0.2	14 ± 1	(1.4 ± 0.2) × 10^5
ampicillin	0.6 ± 0.4	21 ± 3	ND	ND	ND
carbenicillin	2.1 ± 0.1	50 ± 1	ND	ND	ND

[a] Kinetic parameters were determined in 100 mM sodium phosphate, pH 7.0. ND, not determine (for poor turnover).

This procedure produced *S*-(4-butanoate)-cysteine at position 392 of the BlaR1 protein, as a surrogate for the natural N-carboxylated lysine (Scheme 1, panel C). The pK_a of N-carboxylated lysine is 5.2,[12] which is close to that of a carboxylate to make *S*-(4-butanoate)-cysteine as a reasonable mimic of N-carboxylate lysine, except that it cannot experience N-decarboxylation. Determination of the partition ratios for the *S*-(4-butanoate)-cysteine mutant variant revealed that the partition ratios went up in each case (see Table 1), indicative of enhanced turnover of the antibiotics by the modified BlaR1.

We treated the modified BlaR1 as a bona fide enzyme for turnover of ceftazidime, cephalosporin C, cephalothin, and cefepime, the four cephalosporins that were test. The cephalosporins were chosen since their respective partition ratios were enhanced in the approximate range of 90- to 550-fold. In each case we observed saturation, allowing for the measurements of the steady-state kinetic parameters that are tabulated in Table 1. The values for k_{cat}/K_m of 10^4-10^5 M^{-1} s^{-1} typically result in antibiotic resistance as documented for other resistance enzymes[13,14] so this simple alteration of the structure of the receptor protein would appear to be sufficient for manifestation of antibiotic resistance activity.

These evaluations were made under the initial-rate conditions. As the determination of partition ratios indicates, the enzymatic activity is ultimately abrogated. The basis for this observation would appear to be a branched kinetic scheme for the *S*-(4-butanoate)-cysteine variant of BlaR1, a mechanism that is documented with β-lactamases previously.[4,15,16] Maveyraud et al. have documented that the basis for branching of the catalytic scheme in at least one β-lactamase is a conformational change that flips the ester carbonyl of the acyl-enzyme intermediate out of the oxyanion hole that houses it during the catalytic turnover of the substrate.[16] Here, we observe that rapid turnover takes place with β-lactams as substrate for the modified BlaR1, but branching (presumably by the same flipping of the ester carbonyl documented by Maveryraud et al.) brings it to an activity plateau. If the solution is dialyzed at this point and then subjected to the same antibiotic, another cycle of turnover ensues, indicating that the ester carbonyl of the branched species returned to the oxyanion hole and was hydrolytically deacylated to allow restoration of the catalytic activity.

We have shown herein that the mechanistic basis for the BlaR1 protein serving as a receptor is solely its ability to undergo N-decarboxylation of the active site lysine on protein acylation by its ligand. If N-decarboxylation would not take place, the structural template of the protein is endowed to render it into a hydrolytic enzyme. We hasten to add that the mechanism for activation of BlaR1 discussed in this report is unprecedented in the literature for any other protein and it represents a novel strategy by nature in diversification of function from existing structural templates.

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Supporting Information Available: Experimental procedures of cloning, kinetics, and partition ratio determination. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Liu, X. S.; Fan, K.; Wang, W. *Proteins Struct. Funct. Bioinform.* **2004**, *54*, 491−499.
(2) Collins, F. S.; Lander, E. S.; Rogers, J.; Waterston, R. H. *Nature* **2004**, *431*, 931−945.
(3) Birck, C.; Cha, J. Y.; Cross, J.; Schulze-Briese, C.; Meroueh, S. O.; Schlegel, H. B.; Mobashery, S.; Samama, J. P. *J. Am. Chem. Soc.* **2004**, *126*, 13945−13947.
(4) Golemi, D.; Maveyraud, L.; Vakulenko, S.; Samama, J. P.; Mobashery, S. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 14280−14285.
(5) Golemi-Kotra, D.; Cha, J. Y.; Meroueh, S. O.; Vakulenko, S. B.; Mobashery, S. *J. Biol. Chem.* **2003**, *278*, 18419−18425.
(6) Thumanu, K.; Cha, J.; Fisher, J. F.; Perrins, R.; Mobashery, S.; Wharton, C. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 10630−10635.
(7) Li, Z.; Cross, J. B.; Vreven, T.; Meroueh, S. O.; Mobashery, S.; Schlegel, H. B. *Proteins Struct. Funct. Bioinform.* **2005**, *61*, 246−257.
(8) Wilke, M. S.; Hills, T. L.; Zhang, H. Z.; Chambers, H. F.; Strynadka, N. C. J. *J. Biol. Chem.* **2004**, *279*, 47278−47287.
(9) Mobashery, S.; Ghosh, S. S.; Tamura, S. Y.; Kaiser, E. T. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 578−582.
(10) Silverman, R. B. *Mechanism-based enzyme inactivation: Chemistry and Enzymology*; CRC Press: Boca Raton, Fl, 1988; Vol. I, p 9−30.
(11) Toney, M. D.; Kirsch, J. F. *Science* **1989**, *243*, 1485−1488.
(12) Roughton, F. J. W.; Rossi-Bernardi, L. *Carbon dioxide: Chemical, Biochemical and Physiological Aspects*; No. SP-188; NASA: Washington DC, 1970.
(13) Fisher, J. F.; Meroueh, S. O.; Mobashery, S. *Chem. Rev.* **2005**, *105*, 395−424.
(14) Wright, G. D.; Berghuis, A. M.; Mobashery, S. Aminoglycoside antibiotics−Structures, functions, and resistance. In *Resolving The Antibiotic Paradox*; Kluwer Academic: New York, 1998; Vol. 456, pp 27−69.
(15) Citri, N.; Samuni, A.; Zyk, N. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1048−1052.
(16) Maveyraud, L.; Mourey, L.; Kotra, L. P.; Pedelacq, J. D.; Guillet, V.; Mobashery, S.; Samama, J. P. *J. Am. Chem. Soc.* **1998**, *120*, 9748−9752.

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