Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1613–1615

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Binding Interactions of Vancomycin Tracers with a Bacterial Cell Wall Peptidoglycan Analogue

Maciej Adamczyk,* Jonathan Grote, Jeffrey A. Moore, Sushil D. Rege and Zhiguang Yu

Department of Chemistry, Diagnostics Division, Abbott Laboratories, 100 Abbott Park Road, Department 09NM, Bldg. AP-20, Abbott Park, IL 60064-6016, USA

Received 21 April 2000; accepted 18 May 2000

Abstract—Binding interactions between several vancomycin tracers and (*N*,*N*′-diacetyl)KDADA in solution were evaluated in a competition format using a surface plasmon resonance instrument. Tracers derivatized from the carboxy terminus or the *N*-vancosaminyl sugar moiety of vancomycin bind the peptide with an affinity similar to that of underivatized vancomycin. In contrast, *N*-methylleucyl derivatized vancomycin tracers bind the peptide with a reduced affinity relative to vancomycin.

Vancomycin is the most widely used glycopeptide antibiotic for the treatment of Gram-positive infections caused by methicillin resistant *Staphylococcus aureus* and for the treatment of bacterial infections in patients allergic to β-lactam antibiotics.[1,2] For the safe and effective use of the antibiotic, quantification of its levels in patient serum is required to maintain therapeutic levels.[3,4] Immunoassay techniques requiring structurally modified vancomycin tracers are frequently employed for this purpose. Unfortunately, vancomycin is relatively unstable in solution and is readily degraded to an equilibrium mixture (2:3) of biologically inactive crystalline degradation products (CDP-I, Scheme 1). Degradation occurs by rearrangement of the glycopeptide asparagine residue to an isoaspartate residue with concomitant hydrolytic loss of ammonia.[5] Such degradation has lead to problems associated with loss of antibiotic activity and with stability of immunoassay components (i.e., calibrators, controls, and tracers).[6]

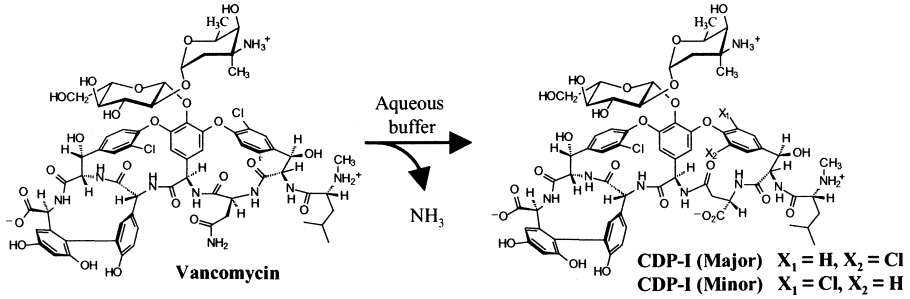
Peptide analogues of the bacterial cell wall have been shown to bind the glycopeptide antibiotic and inhibit the degradation of vancomycin to CDP-I.[7] Thus, binding of vancomycin itself or vancomycin immunoreagents by peptide analogues has been utilized for reagent stabilization. Stabilization is achieved without interference of antibiotic activity or immunoassay reagent performance.[6–8] Significantly, a correlation has been identified between the binding affinity of vancomycin for the peptide analogues and the resulting level of reagent stabilization achieved.[7] We describe here an evaluation of the solution binding interactions between a series of labeled vancomycin tracers developed as potential immunoreagents and a peptide analogue of the bacterial cell wall.

Vancomycin tracers derivatized from the *N*-vancosaminyl carbohydrate, *N*-methylleucyl, or carboxy terminal moieties were prepared as previously described (Table 1).[9] Briefly, *N*-vancosaminyl carbohydrate derivatized tracers were prepared in 81 and 70% yield by reaction of vancomycin in DMF/Et_3N with the NHS active esters of biotin and chemiluminescent 10-(3-sulfopropyl)-*N*-tosyl-*N*-(3-carboxypropyl)-acridinium-9-carboxamide (CPSP-acridinium), respectively. Vancomycin tracers derivatized from the carboxy terminus were prepared in 31 and 35% yield in an identical manner after incorporation of a diaminoalkyl linker from the free carboxyl functionality.[10] Tracers bearing a derivatized *N*-methylleucyl moiety were prepared in 40 and 35% yield, respectively, by coupling vancomycin in DMSO with free biotin or CPSP-acridinium acid in the presence of *N*,*N*′-dicyclohexylcarbodiimide and *N*-hydroxybenztriazole. All vancomycin tracers prepared were purified by preparative HPLC.[9]

Binding interactions between the vancomycin tracers and (*N*,*N*′-diacetyl)KDADA in solution were evaluated in a competition format using a BIAcore surface plasmon resonance instrument.[11,12] Specifically, immobilization of aminocaproate-derivatized (N^{ϵ}-acetyl)KDADA at

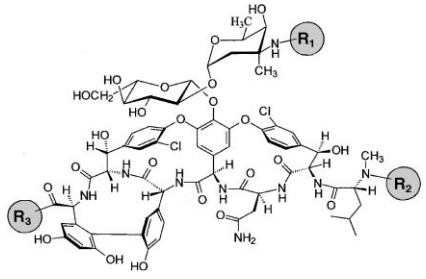
*Corresponding author. Tel.: +1-847-937-0225; fax: +1-847-938-8927; e-mail: maciej.adamczyk@abbott.com

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00298-5



Scheme 1.

Table 1. Structures of vancomycin tracers



Tracer	R_1	R_2	R_3
N-vancosaminyl-derived	Label[a]	H	OH
N-methylleucyl-derived	H	Label	OH
Carboxyl-HDA-derived	H	H	$NH(CH_2)_6NH$-Label

[a]Label = biotin or CPSP-acridinium.

different surface densities on two flow cells of a CM-5 sensor chip (BIAcore, Inc.) provided a gradient surface that was utilized to correlate binding response with a concentration of free vancomycin tracer in solution.[9,13] Several known concentrations of each vancomycin tracer were initially injected over these sensor surfaces. The resulting instrumental response from the lower density aminocaproate-derivatized (N^{ε}-acetyl)KDADA surface was subtracted from the higher density surface to eliminate bulk solvent effects and any nonspecific binding events. The true binding response (RU) for each concentration of tracer injected was then determined by averaging the response observed between 215–230 s postinjection for the corrected sensorgram (Fig. 1A). A plot of RU versus the concentration of antibiotic tracer injected was subsequently fit using a 4-parameter logistic (general model, BIAevaluation 3.0) providing a standard curve for each analogue evaluated (Fig. 1B).

A fixed concentration of each vancomycin tracer was then mixed with several concentrations of soluble (*N*,*N*′-diacetyl)KDADA and the solutions were allowed to reach equilibrium. The equilibrium mixtures were individually injected over the aminocaproate-derivatized (N^{ε}-acetyl)KDADA sensor surfaces, and the concentration of free vancomycin tracer remaining in the solution at equilibrium was quantitated by determination of the binding response from the corrected sensorgrams as described above and the appropriate standard curve (Fig. 2A). A plot of free vancomycin tracer versus total concentration of added (*N*,*N*′-diacetyl)KDADA provided a competition curve (Fig. 2B). A fit of the data sets utilizing the solution affinity model in BIAevaluation software (v3.0) yielded equilibrium dissociation constants for the binding interactions between each vancomycin tracer and (*N*,*N*′-diacetyl)KDADA in solution.[12]

Equilibrium dissociation constants for the solution binding interactions between vancomycin tracers and (*N*,*N*′-diacetyl)KDADA determined using the competition format described here are summarized in Table 2. As a reference, the binding interaction of underivatized vancomycin with the same tripeptide determined using the

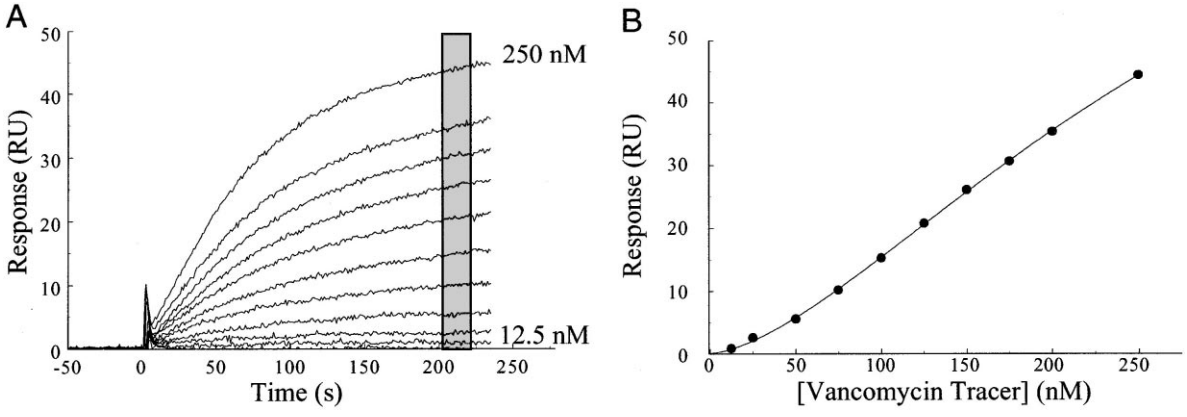


Figure 1. (A) Representative overlay plot of corrected sensorgrams generated for a vancomycin tracer binding to the aminocaproate-derivatized (N^{ε}-acetyl)KDADA sensor surface. Data shown is for the carboxyl-HDA-acridinium labeled vancomycin tracer (12.5–250 nM). Binding responses were obtained from the shaded portion of each sensorgram. (B) Standard curve obtained for binding of the carboxyl-HDA-acridinium labeled vancomycin tracer to the aminocaproate-derivatized (N^{ε}-acetyl)KDADA sensor surface.

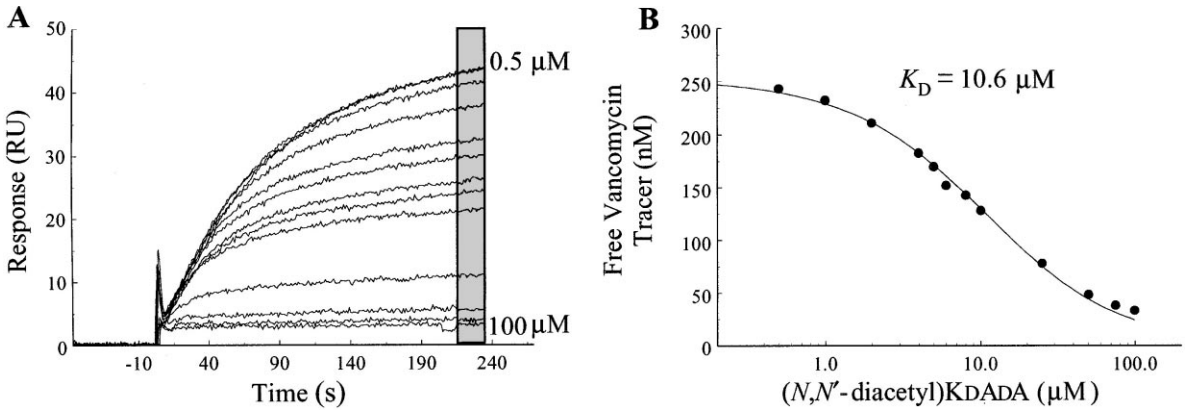


Figure 2. (A) Representative overlay plot of corrected sensorgrams generated from a study of vancomycin tracer/tripeptide binding interactions in a competition format. Data shown is for the carboxyl-HDA-acridinium labeled vancomycin tracer competition study (250 nM tracer, 0.5–100 μM tripeptide). Binding responses were obtained from the shaded portion of each sensorgram. (B) Nonlinear regression plot of free carboxyl-HDA-acridinium labeled vancomycin tracer vs total concentration of added (*N*,*N*′-diacetyl)KDADA.

Table 2. Summary of the equilibrium dissociation constants for binding interactions of vancomcycin tracers with (*N*,*N*′-diacetyl) KDADA determined utilizing the BIAcore solution competition format

	Derivatization site		
Derivative	*N*-vancosaminyl	*N*-methylleucyl	Carboxyl-HDA
Acridinium	5.4 μM	86 μM	10.6 μM
Biotin	3.7 μM	168 μM	4.8 μM

competition format provided an equilibrium dissociation constant of 2.4 μM. The data shows tracers derivatized from the *N*-vancosaminyl sugar moiety or the carboxy terminus of vancomycin bind (*N*,*N*′-diacetyl)KDADA with affinities similar to that of underivatized vancomycin. These equilibrium dissociation constants are consistent with literature values reported for vancomycin/tripeptide analogue binding interactions in solution.[14–16] In contrast, tracers derivatized from the *N*-methylleucyl moiety of vancomycin exhibit a reduced affinity for the tripeptide analogue relative to vancomycin. The reduced binding interaction indicates a disruption of the known electrostatic interaction between the *N*-methylleucyl moiety of vancomycin and the carboxy terminus of the tripeptide.[16] The influence such differences in the vancomycin tracer/tripeptide binding affinity play in terms of reagent stabilization and ultimately assay performance are currently being investigated as well as an investigation into the potential use of these vancomycin compounds as "chemical probes" for Gram-positive bacteria.

References

1. Kirby, W. M. M. *Rev. Infect. Dis.* **1981**, *3*, 5236.
2. Ingerman, M. J.; Santoro, J. *Infect. Dis. Clin. North. Am.* **1989**, *3*, 641.
3. Pryka, R. D.; Rodvold, K. A.; Erdman, S. M. *Clin. Pharmacokinet.* **1991**, *20*, 463.
4. Ryback, M. J.; Boike, S. C. *Drug Intell. Clin. Pharm.* **1986**, *20*, 757.
5. Harris, C. M.; Kopecka, H.; Harris, T. M. *J. Am. Chem. Soc.* **1983**, *105*, 6915.
6. Morishige, H.; Shuto, H.; Ieiri, I.; Otsubo, K.; Ryozo, O. *Ther. Drug Monit.* **1996**, *18*, 80.
7. Harris, C. M.; Kopecka, H.; Harris, T. M. *J. Antibiot.* **1985**, *38*, 51.
8. Adamczyk, M.; Brate, E. M.; Chiappetta, E. G.; Ginsburg, S.; Hoffman, E.; Klein, C.; Perkowitz, M. M.; Rege, S. D.; Chou, P. P.; Constantino, A. G. *Ther. Drug Monit.* **1998**, *20*, 191.
9. Adamczyk, M.; Grote, J.; Moore, J. A.; Rege, S. D.; Yu, Z. *Bioconjugate Chem.* **1999**, *10*, 176.
10. Sundram, U. N.; Griffin, J. H. *J. Org. Chem.* **1995**, *60*, 1102.
11. Karlsson, R. *Anal. Biochem.* **1994**, *221*, 142.
12. Adamczyk, M.; Moore, J. A.; Yu, Z. *Methods* **2000**, *20*, 319.
13. Karlsson, R.; Ståhlberg, R. *Anal. Biochem.* **1995**, *228*, 274.
14. Nieto, M.; Perkins, H. R. *Biochem. J.* **1971**, *123*, 773.
15. Rao, J.; Colton, I. J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1997**, *119*, 9336.
16. Kannan, R.; Harris, C. M.; Harris, T. M.; Waltho, J. P.; Skelton, N. J.; Williams, D. H. *J. Am. Chem. Soc.* **1988**, *110*, 2946.