Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 18, pp. 2151-2156, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00366-6

α-VINYLLYSINE AND α-VINYLARGININE ARE TIME-DEPENDENT INHIBITORS OF THEIR COGNATE DECARBOXYLASES

David B. Berkowitz* Wan-Jin Jahng and Michelle L. Pedersen

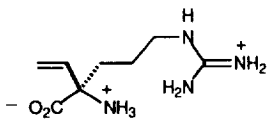
Department of Chemistry, University of Nebraska-Lincoln, Lincoln, NE 68588-0304

Abstract: (±)-α-Vinyllysine and (±)-α-vinylarginine display time-dependent inhibition of L-lysine decarboxylase from *B. cadaveris*, and L-arginine decarboxylase from *E. coli*, respectively. A complete Kitz-Wilson analysis has been performed using a modification of the Palcic continuous UV assay for decarboxylase activity.

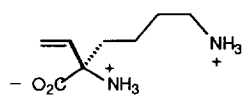
As part of a program directed at the synthesis of new, α-branched amino acids, we have previously described a convenient method for the synthesis of α-vinyl amino acids,[1a,b] and for their enzymatic resolution.[1c] We have also established that α-vinyl amino acids are useful precursors to the corresponding α-chlorovinyl,[1d] α-bromovinyl[1d] and α-oxiranyl amino acids.[1e] Herein, we provide a detailed kinetic characterization of two new time-dependent inhibitors of the α-vinyl amino acid class.

Several α-vinyl amino acids are known to act as mechanism-based inhibitors for pyridoxal phosphate dependent enzymes. The simplest α-vinyl amino acid, α-vinylglycine, is a natural product[2] and inactivates transaminases for L-aspartate, L-alanine, L-serine and D-alanine.[3a-e] One decarboxylase, L-cysteine sulfinate decarboxylase, is also irreversibly inactivated by α-vinylglycine.[3f] Peptidylglycine α-hydroxylating monooxygenase (PHM) is irreversibly inhibited by a D-α-vinylglycine-containing tripeptide.[4]

The higher vinyl amino acids (with R groups more complex than hydrogen) are not known natural products, though a considerable number have been synthesized.[1a,5] Previous work has shown that DOPA decarboxylase,[6] ornithine decarboxylase[7] and glutamate decarboxylase,[8] all important medicinal targets, are irreversibly inhibited by the corresponding (±)-α-vinyl amino acids. In the cases of α-vinylhistidine[6a] and α-vinylserine[9] (reversible) competitive inhibition is observed with the PLP-dependent histidine decarboxylase and with serine hydroxymethyltransferase, respectively. A related vinyl amino acid, γ-vinyl-GABA (Vigabatrin) is a time-dependent inhibitor of GABA transaminase and an anti-epileptic drug.[10] Elegant studies by Silverman and coworkers have provided useful insights into the mechanism of action of this γ-vinyl-γ-amino acid.[11]



(±) **1**



(±) **2**

Using our formal vinylation procedure,[1a] we synthesized (±)-α-vinylarginine (**1**) and (±)-α-vinyllysine (**2**) as potential mechanism-based inhibitors of L-arginine decarboxylase (ADC) and L-lysine decarboxylase (LDC), respectively. Indeed, several other α-branched (primarily α-halogenomethyl) amines and amino acids

have been developed by others as mechanism-based inhibitors of ADC[12] and LDC.[13] These two amino acid decarboxylases are important targets for the development of antiproliferative agents as their reaction products feed into the polyamine pathway. The ADC reaction product, agmatine, is converted to putrescine via agmatine amidinohydrolase (agmatinase; EC 1.5.3.11). The LDC reaction product, cadaverine, is used as a putrescine-surrogate in a modified polyamine pathway found in tumor cells that have been exposed to ornithine DC inhibitors.[14] ADC inhibitors are also potential antibiotics as this enzyme is found in bacterial and plants, but not in mammalian systems.[12]

Time-Dependent Inhibition Kinetics

We wished to use a continuous UV assay to evaluate (±)-α-vinylarginine and (±)-α-vinyllysine for time-dependent inhibition of their cognate decarboxylases in contrast to the radioactive, time-point assay that has been traditionally used.[6-8] We chose to develop a modified version of the assay described by Palcic and coworkers.[15] In this assay, the CO_2 formed upon decarboxylation is trapped by phosphoenolpyruvate carboxylase (PEPC)-mediated condensation with PEP to produce oxaloacetate. Subsequent in situ reduction of the oxaloacetate to L-malate with malate dehydrogenase results in a decrease in $O.D._{340}$ due to the oxidation of NADH to NAD^+. Whereas Palcic used a CO_2 kit for this assay, we chose to assemble each of the individual components, and in so doing, to optimize the assay in terms of pH and source and amount of each enzyme, in particular. Our optimal conditions are presented below. These assay conditions were first tested for each enzyme by carrying out a standard Lineweaver-Burk steady state kinetic analysis with the natural substrate. In this way, we obtained K_m = 180 μM for L-arginine with arginine decarboxylase (Figure 1) and K_m = 640 μM for L-lysine with lysine decarboxylase (Figure 2).

Optimized Continuous UV Assay for Decarboxylase Activity:

General. The assay cuvet (total volume 1 mL) contained 80 mM potassium phosphate buffer (pH 6.8), 2 mM L-lysine, 0.4 mM PLP, 0.01 U lysine DC (*Bacterium cadaveris*, 55 U/mg protein, single band on SDS-PAGE), 2 mM PEP, 10 mM $MgCl_2$, 0.9 U PEPC (*Zea mays*), 0.04 U carbonic anhydrase, 1.5 U malate dehydrogenase, 0.21 mM NADH.

Time-dependent inhibition assays. E_t cuvet: the enzyme was incubated with (±)-α-vinyllysine and all other components with the exception of lysine. E_o cuvet: A control cuvet identical to the E_t cuvet, but lacking inhibitor, was incubated under the same conditions. After incubation of both cuvets at 25° C for a time t, background rates for each cuvet were measured by monitoring $\Delta O.D._{340}$ vs. time.* L-lysine (2 mM) was then added to each cuvet and initial rates were measured as before. For primary plots, E_t/E_o was calculated as follows: $E_t = v_o[(LDC + I) + L\text{-}lys]_t - v_o(LDC + I)_t$ and $E_o = v_o(LDC + L\text{-}lys)_t - v_o(LDC)_t$.

For arginine DC (*E. Coli.*, 215 U/mg protein, single band on SDS-PAGE), the same procedure was followed except that all incubations and assays were run at pH 6.5.

*This is necessary because there is always a small background rate due to exogenous CO_2 in the control cuvet. For the assay cuvet, there is an additional, albeit small, component of the background rate due to residual α-vinyllysine decarboxylation, which must be subtracted out to obtain the true rate of lysine decarboxylation. Background rates were ≤10% of v_o.

Figure 1

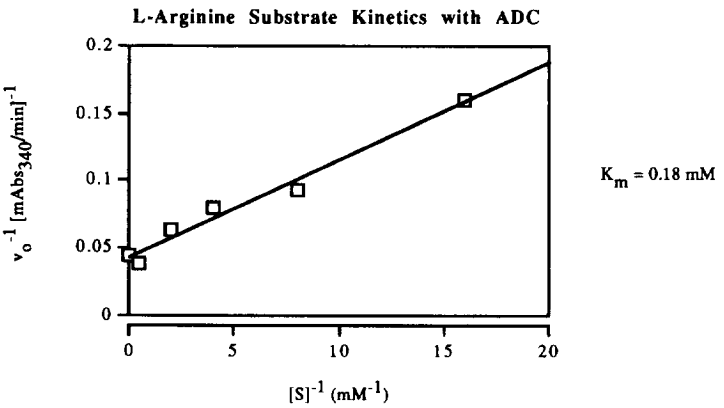
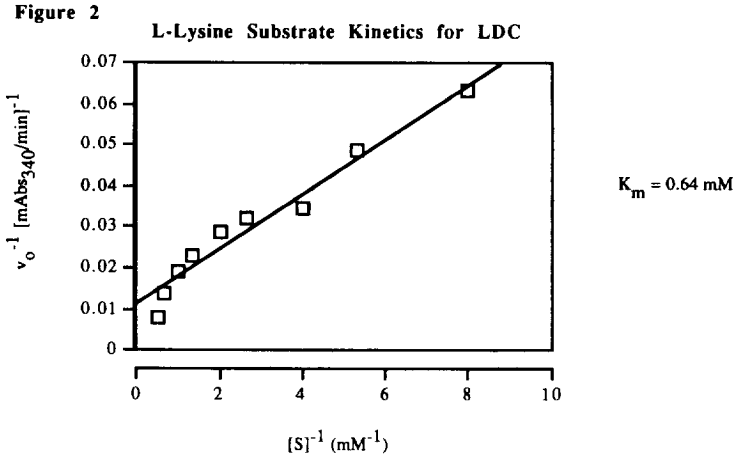


Figure 2



Then, for each inhibitor and each enzyme, a complete Kitz-Wilson analysis[16] was performed. This involves pre-incubating a given decarboxylase with a fixed concentration of its cognate α-vinyl amino acid inhibitor candidate (vide supra). The slope of the primary plot {$\ln(E/E_o)$ vs. time of incubation} corresponds to an apparent inactivation constant for that inhibitor concentration. After repeating the experiment at several inhibitor concentrations, a secondary plot {reciprocal k_{inact}(apparent) versus 1/[I]} is constructed, the intercept and slope of which provide k_{inact} and K_I, respectively:

Primary Plot: $\ln(E/E_o) = -[k_{inact}/(1 + K_I/[I])]t$

Secondary Plot: $-1/\text{primary slope} = 1/k_{inact}(\text{apparent}) = (K_I/k_{inact})(1/[I]) + 1/k_{inact}$

Figure 3

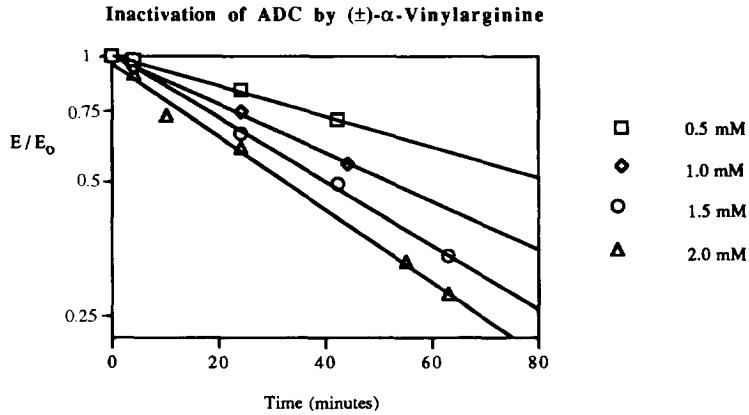
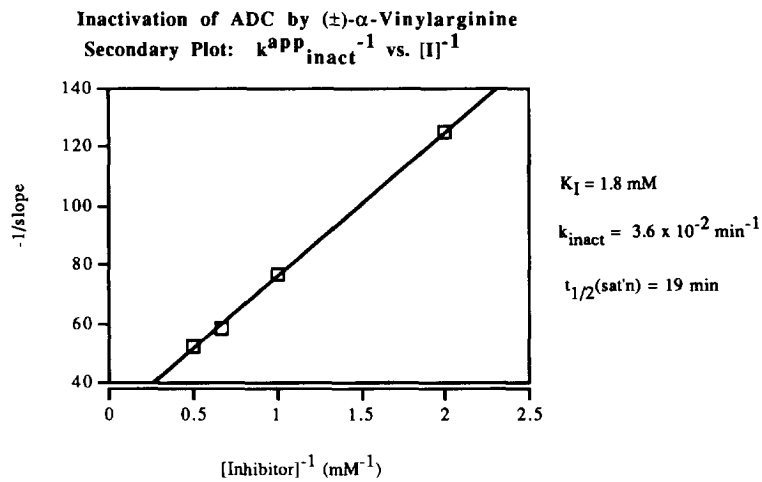


Figure 4



As can be seen from Figures 3-6 both (±)-α-vinylarginine and (±)-α-vinyllysine exhibit well-behaved time-dependent inhibition of their cognate DC's. We obtain, K_I = 1.8 mM and k_{inact} = 3.6 x 10^{-2} min^{-1} for (±)-α-vinylarginine with L-arginine DC. These data indicate a relatively weak affinity of this enzyme for this α-branched alternative substrate, though the rate of inactivation is quite respectable. On the other hand, (±)-α-vinyllysine displays K_I = 0.5 mM and k_{inact} = 2 x 10^{-2} min^{-1} with L-lysine DC. While the observed first order inactivation rate constant here is marginally smaller than that observed for (±)-α-vinylarginine with arginine DC, the K_I is respectable. If one considers that the enzyme probably turns over only one enantiomer (subject to future verification) of this apparent Trojan horse inhibitor, the K_I is actually about 250 μM, which is nearly a factor of three smaller than K_m. This indicates that an α-vinyl substituent is particularly well-tolerated by LDC. Furthermore, dialysis of inhibited LDC against several changes of buffer (conditions under which LDC activity does not decrease) fails to restore any LDC activity, indicating that the observed inhibition is irreversible.

Figure 5 **Inactivation of LDC by (±)-α-Vinyllysine**

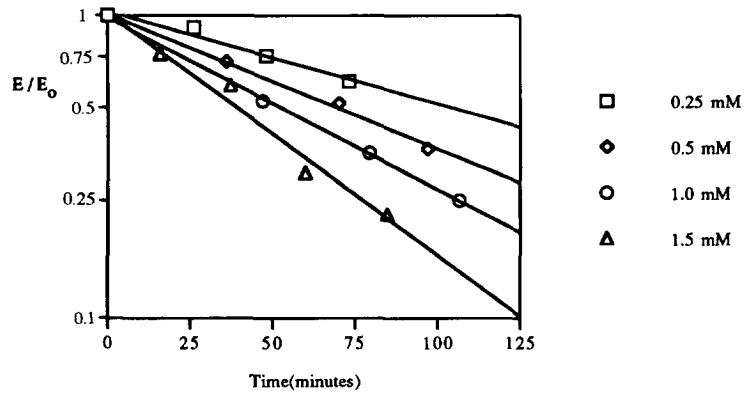
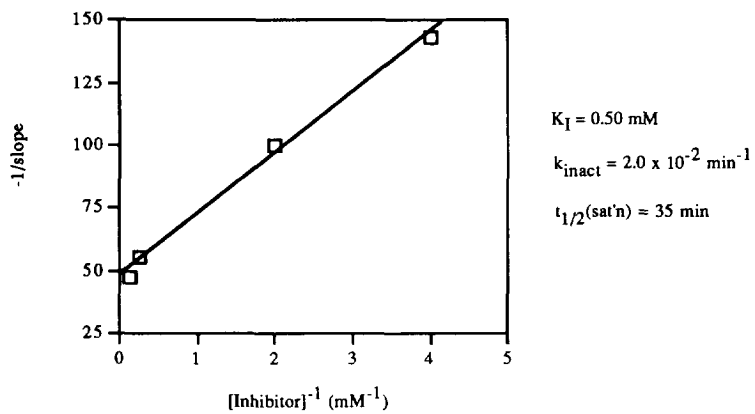


Figure 6

Inactivation of LDC by (±)-α-Vinyllysine

Secondary Plot: $k^{app}{}_{inact}{}^{-1}$ vs. $[I]^{-1}$



Summary

Using our procedure for the formal α-vinylation of α-amino acids, (±)-α-vinylarginine and (±)-α-vinyllysine were synthesized. A modification of the continuous UV assay for decarboxylases reported by Palcic was used to conveniently perform a Kitz-Wilson kinetic analysis for time-dependent inhibition with these α-branched amino acids. Indeed, both compounds display pseudo-first order time-dependent inhibition of their respective amino acid decarboxylases, with (±)-α-vinyllysine displaying a K_I that compares favorably with K_m.

Acknowledgment.

Financial support from the National Institutes of Health (CA 62034) is gratefully acknowledged. This research was facilitated by grants for shared instrumentation (NMR and GC/MS) from the NIH (SIG 1-S10-RR06301) and the NSF (CHE-93000831), respectively.

References.

1. (a) Pedersen, M. L.; Berkowitz, D. B. *J. Org. Chem.* **1993**, *58*, 6966-6975; (b) Berkowitz, D. B.; Smith, M. K. *Synthesis* **1996**, 39-41; (c) Berkowitz, D. B.; Pumphrey, J. A.; Shen, Q. *Tetrahedron Lett.* **1994**, *35*, 8743-8746; (d) Berkowitz, D. B.; Pedersen, M. L.; Jahng, W. J. *Tetrahedron Lett.* **1996**, 37, 4309-4312; (e) Berkowitz, D. B.; Pedersen, M. L. *J. Org. Chem.* **1995**, *60*, 5368-5369.
2. Dardenne, G.; Casimir, J.; Marlier, M.; Larsen, P. O. *Phytochemistry* **1974**, *13*, 1897-1900.
3. (a) Rando, R. R.; Relyea, N.; Cheng, L. *J. Biol. Chem.* **1976**, *251*, 3306-3312; (b) Rando, R. R. *Biochemistry* **1974**, *13*, 3859-3863; (c) Soper, T. S.; Manning, J. M.; Marcotte, P. A.; Walsh, C. T. *J. Biol. Chem.* **1977**, *252*, 1571-1575; (d) Cornell, N. W.; Zuurendonk, P. F.; Kerich, M. J.; Straight, C. B. *Biochem. J.* **1984**, *220*, 707-716; (e) Cho, C.; Ishii, R.; Hyeon, S.; Suzuki, A. *Agric. Biol. Chem.* **1987**, *51*, 2597-2598.; (f) Griffith, O. W. *J. Biol. Chem.* **1983**, *258*, 1591-1598.
4. Zabriskie, T. M.; Cheng, H.; Vederas, J. C. *J. Am. Chem. Soc.* **1992**, *114*, 2270-2272.
5. (a) Colson, P.-J.; Hegedus., L. S. *J. Org. Chem.* **1993**, *58*, 5918-5924; (b) Seebach, D.; Bürger, H. M.; Schickli, C. P. *Liebigs Ann. Chim.* **1991**, 669-684; (c) Castelhano, A. L.; Horne, S.; Taylor, G. J.; Billedeau, R.; Krantz, A. *Tetrahedron*, **1988** *44*, 5451-5466; (d) Münster, P.; Steglich, W. *Synthesis* **1987**, 223-225; (e) Weber, T.; Aeschimann, R.; Maetzke, T.; Seebach, D. *Helv. Chim. Acta.* **1986**, *69*, 1365-1377; (c) Castelhano, A. L.; Horne, S.; Billedeau, R.; Krantz, A. *Tetrahedron Lett.* **1986**, *27*, 2435-2438; (d) Steglich, W.; Wegmann, H. *Synthesis* **1980**, 481-483; (e) Metcalf, B. W.; Bonilavri, E.; *J. Chem. Soc. Chem. Commun.* **1978**, 914-915; (f) Greenlee, W. J.; Taub, D.; Patchett, A. A. *Tetrahedron Lett.* **1978**, 3999-4002; (g) Metcalf, B. W.; Jund, K. *Ibid.* **1977**, 3689-3692; (h) Taub, D.; Patchett, A. A. *Ibid.* **1977**, 2745-2748.
6. (a) Maycock, A. L.; Aster, S. D.; Patchett, A. A. *Developments in Biochemistry* **1979**, *6*, 115-129; (b) Ribereau-Gayon, G.; Danzin, C.; Palfreyman, M. G.; Aubry, M.; Wagner, J.; Metcalf, B. W.; Jung, M. *J. Biochem. Pharm.* **1979**, *28*, 1331-1335; (c) Maycock, A. L.; Aster, S. D.; Patchett, A. A. in *Enzyme-Activated Irreversible Inhibitors* Seiler, N.; Jung, M. J.; Koch-Weser, J., Eds. (Elsevier, North Holland, 1978) pp. 211-220.
7. Danzin, C.; Casara, P.; Claverie, N.; Metcalf, B. W. *J. Med. Chem.* **1981**, *24*, 16-20.
8. Metcalf, B.; Jung, M. U.S. Patent 4,147,873; April 3, 1979.
9. Tendler, S.J.B.; Threadgill, M.D.; Tisdale, M.J. *J. C. S. Perkin Trans. I* **1987**, 2617-2623.
10. Jung, M. J.; Palfreyman, M. G. *Vigabatrin. Mechanisms of Action* in *Antiepileptic Drugs*, 4th Ed. R. H. Levy; R. H. Mattson, B. S. Meldrum, Eds. (Raven, New York, 1995) pp. 903-913.
11. Nanavati, S.; Silverman, R. B. *J. Am. Chem. Soc.* **1991**, *113*, 9341-9349.
12. (a) Christ, M.; Felix, H.; Harr, J. *Z. Naturforsch.* **1989**, *44c*, 49-54; (b) Felix, H.; Harr, J. *Ibid.* **1989**, *44c*, 55-58; (c) Bitonti, A. J.; Casara, P. J.; McCann, P. P.; Bey, P. *Biochem J.* **1987**, *242*, 69-74; (d) Kallio, A.; McCann, P. P.; Bey, P. *Biochem.* **1981**, *20*, 3163-3166.
13. (a) Yamamoto, S.; Imamura, T.; Kusaba, K.; Shinoda, S. *Chem. Pharm. Bull.* **1991**, *39*, 3067-3070; (b) Poso, H.; McCann, P. P.; Tanskanen, R.; Bey, P.; Sjoerdsma, A. *Biochem. Biophys. Res. Commun.* **1984**, *125*, 205-210.
14. Alhonen-Hongisto, L.; Seppanen, P.; Holtta, E.; Janne, J. *Biochem. Biophys. Res. Commun.* **1982**, *106*, 291-297.
15. Scriven, F.; Wlasichuk, K. B.; Palcic, M. M. *Anal. Biochem.* **1988**, *170*, 367-371.
16. Kitz, R.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245-3249.

(*Received in USA* 26 *June* 1996; *accepted* 25 *July* 1996)