Tetrahedron Letters,Vol.23,No.31,pp 3139-3142,1982 0040-4039/82/313139-04$03.00/0
Printed in Great Britain

STEREOCHEMISTRY OF THE BACTERIAL ORNITHINE, LYSINE, AND ARGININE DECARBOXYLASE REACTIONS

Gary R. Orr and Steven J. Gould*,[1]

School of Pharmacy, Section of Medicinal Chemistry and Pharmacognosy,
University of Connecticut, Storrs, Connecticut 06268

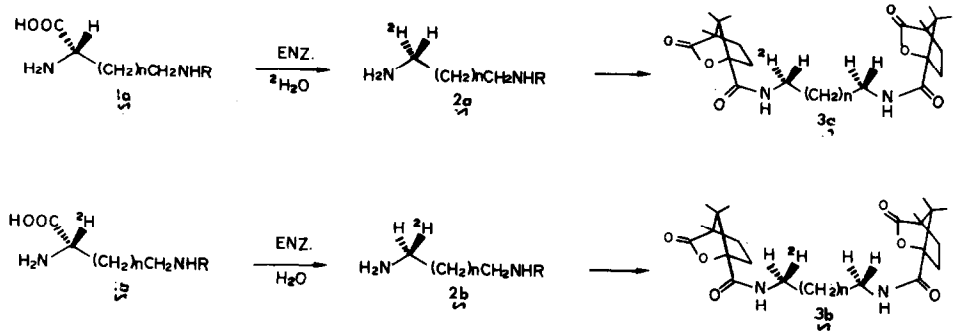
Abstract: The stereochemistry of the bacterial lysine, ornithine, and arginine decarboxylase reactions is determined by ^{1}H-NMR analysis.

Amino acid decarboxylases, a metabolically important class of enzymes, are known for a wide variety of substrates and can be divided into two subclasses based on their coenzyme requirement: either pyridoxal phosphate or pyruvate.[2] As part of our study of stereochemistry in polyamine biosynthesis[3] we examined the ornithine decarboxylase reaction, and have extended our work to include decarboxylases for two other basic amino acids: lysine and arginine. These decarboxylases use the more common pyridoxal phosphate coenzyme. While the lysine decarboxylase (LDC) from Bacillus cadaveris has been studied using tritium labels,[4] the low yields obtained and the danger of hydrogen exchange in the strong acid medium for the chemical degradation utilized limit the potential usefulness of this approach.

We have chosen to study ornithine decarboxylase (ODC) from E. coli, LDC from B. cadaveris and from E. coli, and arginine decarboxylase (ADC) from E. coli ATCC 10787.[5] The stereochemistry of the stereospecifically deuterated symmetrical diamines ultimately obtained by the action of these enzymes was determined by a simple NMR analysis in the presence of a Europium shift reagent. The ^{1}H NMR signals for the diastereotopic hydrogens of the methylene adjacent to nitrogen in the methyl esters of γ-aminobutyric acid camphanamide[6] and glycine camphanamide[7] have been shown to be separable in the presence of [Eu (dpm)$_3$]. We have found this also to be true for the symmetrical biscamphanamides of cadaverine and putrescine; we also found that [Eu (fod)$_3$] gives substantially better separations at lower molar %.

For each decarboxylase two enantiomeric monodeuterated diamines were prepared. One (2a) was obtained by carrying out the reaction in buffer prepared with D_2O, while the epimer (2b) was obtained by decarboxylation of α-deutero amino acid[8] (1b) in buffer prepared with H_2O.[9] For ADC, the deuterated agmatines initially produced were hydrolyzed with H_2SO_4 to give the deuterated putrescines.[10] Each biscamphanamide (3) was prepared with camphanoyl chloride,[11] recrystallized to purity,[12] and its deuterium content measured by mass spectrometry. Proton NMR spectra in the presence of 10 mole % [Eu (fod)$_3$] were obtained at 270 MHz (biscamphanamides from B. cadaveris) and at 500 MHz (all the rest); chemical shifts and signal integrations are given in Table 1. The

1R- and 1S-signals of the cadaverine and putrescine derivatives were well separated; for the products of all three enzymes it was clear that deuterium was located at the downfield signal when the enzyme substrate had been a deuterated amino acid and was located in the upfield signal when deuterium had been introduced from the reaction medium.



Scheme 1. Lysine; n=3, R=H. Ornithine; n=2, R=H. Arginine; n=2, R=amidino.

Gerlach and Zagalak[13] had noted an empirical correlation indicating that in the presence of [Eu (dpm)$_3$] the pro S-hydrogen ^{1}H NMR signal of camphanate esters of several primary alcohols occurred at lower field than the pro-R hydrogen signal. This correlation was found to hold as well for the camphanamides of γ-aminobutyric acid methyl ester[6] and of glycine methyl ester,[7] and these assignments were established in an absolute sense by unambiguous chiral synthesis. Based on this correlation, our results indicate that all three enzyme reactions introduce hydrogen at the pro-R position, thus proceeding with retention of configuration. In the case of B. cadaveris LDC, this is consistent with Spenser's findings.[4]

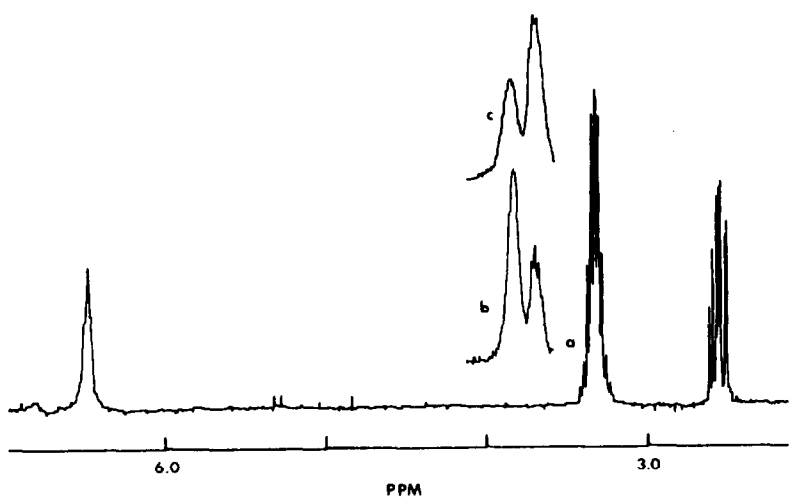


Figure 1. a) 270 MHz ^{1}H NMR spectrum of unlabeled cadavarine biscamphanamide. b) methylene signal of R-isomer in presence of 10 mole % [Eu (fod)$_3$]. c) same as b for S isomer.

In order to firmly establish the absolute configurations of our deuterated diamines the cadaverine hydrochlorides from B. cadaveris LDC were each carefully oxidized to deuterated glycines,[14] and the NMR spectra of the camphanamide methyl esters obtained.[7] Indeed, it is the pro-S hydrogen signal of the biscamphanamide that appears at lower field.

For further biosynthetic studies it would be advantageous to extend this approach to the diaminopropane (DAP) unit. We prepared the deuterated γ-aminobutyric acids by decarboxylation of glutaminic acid with glutamate decarboxylase[6] and converted each to a DAP in 30-35% yield by Schmidt degradation. However, in the case of these biscamphanamides[15] no chemical shift separation was obtained even at high mole % [Eu $(fod)_3$] Repetition of the experiment using the weaker Lewis acid [Eu $(dpm)_3$] at slightly elevated temperature also failed to afford any resolution of the diastereotopic signals.

We have now presented evidence that establishes that the bacterial ODC, LDC, and ADC reactions all occur with retention of configuration. Thus, all amino acid decarboxylases so far studied, including those that require pyridoxal phosphate[16] and those that require pyruvate,[17] proceed in the same stereochemical sense.

TABLE 1

Substrate	Medium	Enzyme	Chem. Shift (δ) of α-H (int)	% $^2H^a$	Integration of α-H[b] downfield	upfield	Config.	Stereochem. of rx
[2-^{2}H]-lys	H_2O	LDC B. subt.	3.30 (3.5 H)	47	1.5 H	2.0 H	S	Retention
L-lys	D_2O	LDC B. subt.	3.30 (3.0 H)	94	2.0 H	1.0 H	R	Retention
[2-^{2}H]-lys	H_2O	LDC E. coli	3.30 (3.2 H)	79	1.2 H	2.0 H	S	Retention
L-lys	D_2O	LDC E. coli	3.30 (3.0 H)	91	2.0 H	1.0 H	R	Retention
[2-^{2}H]-orn	H_2O	ODC E. coli	3.32 (3.2 H)	78	1.2 H	2.0 H	S	Retention
L-orn	D_2O	ODC E. coli	3.32 (3.2 H)	91	2.0 H	1.2 H	R	Retention
[2-^{2}H]-arg	H_2O	ADC E. coli	3.32 (3.3 H)	67	1.3 H	2.0 H	S	Retention
L-arg	D_2O	ADC E. coli	3.32 (3.1 H)	93	2.0 H	1.1 H	R	Retention

a. determined by mass spectroscopy

b. in presence of 10% [Eu $(fod)_3$]

Acknowledgments

This work was supported by grants to Steven J. Gould from the National Science Foundation (PCM 7615197) and the National Institutes of General Medical Sciences (GM 28990). NMR spectra were obtained on the Bruker HX 270 and WM 500 spectrometers at the Northeast Regional NSF-NMR Facility, Yale University, partially supported by the National Science Foundation, Grant CHE 79-16210.

References

1. Career Development Awardee of the National Cancer Institute (CA00627), 1979-1984.
2. E.A. Boeker and E.E. Snell in The Enzymes, 3rd. Ed., P.D. Boyer, ed., vol. 6, Acad. Press, New York, 1972, pp. 217-253.
3. U. Bachrach, Function of the Naturally Occurring Polyamines, Acad. Press, New York, 1973.

4. E. Leistner and I.D. Spenser, JCS Chem. Commun., 378 (1975).
5. Obtained from Sigma Chemical Co. This strain lacks ornithine decarboxylase activity. Enzymatic reactions were carried out according to published procedures.
6. E. Santaniello, M.G. Kienle, A. Manzocchi, and E. Bosisio, J. Chem. Soc. Perkin I., 1677 (1979).
7. W.L.F. Armarego, B.A. Milloy and W. Pendergast, J. Chem. Soc. Perkin I., 2229 (1976).
8. Inventa Akt. - Ges. fur Forschung und Patentverwertung, British Patent 847, 785 (1960); Chem. Abstr. 55:P8308C (1960).
9. Enzymatic reactions run on a 0.5-2.0 millimolar scale.
10. Separate experiments demonstrated that no putrescine was formed enzymatically under the conditions employed.
11. Procedure modified from that published by Armarego ref. 7.
12. Unlabeled compounds were characterized by IR, NMR and high resolution mass spectroscopy.
13. H. Gerlach, and B. Zagalak, JCS Chem. Commun., 274 (1973).
14. Conditions personally communicated to S.J. Gould by I.D. Spenser.
15. Mass spectroscopic analysis indicated that the R & S isomers contained 93 and 75% deuterium respectively.
16. L-Lysine decarboxylase: (a) ref. 4; (b) H.J. Gerdes, and E. Leistner, Phytochemistry 18, 771 (1979). L-tyrosine decarboxylase: (a) B. Belleau and J. Burba, J. Am. Chem. Soc., 82, 5751 (1960); (b) A.R. Battersby, E.J.T. Chrystal, and J. Staunton, J. Chem. Soc. Perkin Trans. 1, 31 (1980). L-glutamic acid decarboxylase: (a) H. Yamada and M.H. O'Leary, Biochemistry, 17, 669 (1978); (b) ref. 6. L-histidine decarboxylase: (a) A.R. Battersby, R. Joyeau and J. Staunton, FEBS Lett., 107, 231 (1979); (b) A.R. Battersby, N. Nicoletti, J. Staunton, and R. Vleggaar, J. Chem. Soc. Perkin Trans. 1, 43 (1980).
17. S-adenosyl methionine decarboxylase: R.R. Allen, and J.P. Klinman, J. Biol. Chem., 256, 3233 (1981). L-histidine decarboxylase (bacterial) A.R. Battersby, N. Nicoletti, J. Staunton and R. Vleggaar, J. Chem. Soc. Perkin Trans. 1, 43 (1980).

(Received in USA 10 May 1982)