Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 929–931

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Probing the Overlap of Chorismate Mutase and Prephenate Dehydrogenase Sites in the *Escherichia coli* T-Protein: A Dehydrogenase-Selective Inhibitor

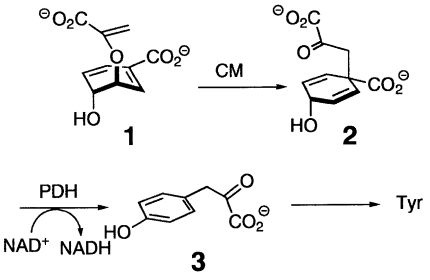
Sarah Vincent,[a] Shuqing Chen,[b] David B. Wilson[b] and Bruce Ganem[a,*]

[a]*Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA*
[b]*Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853, USA*

Received 12 December 2001; accepted 14 January 2002

Abstract—An inhibitor of prephenate dehydrogenase has been identified that has no effect on the chorismate mutase activity in the *Escherichia coli* T-protein, thus supporting the idea of two separate active sites. © 2002 Elsevier Science Ltd. All rights reserved.

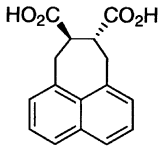
Tyrosine (Tyr) is biosynthesized in *Escherichia coli* and other enteric bacteria from *p*-hydroxyphenylpyruvate (**3**, Scheme 1), In *E. coli,* **3** is produced from chorismic acid **1** by the action of the bifunctional T-protein, which exhibits chorismate mutase (CM) and prephenate dehydrogenase (PDH) activities.[1] An analogous biosynthetic pathway to phenylalanine (Phe) in *E. coli* utilizes the bifunctional P-protein, which consists of CM in tandem with prephenate dehydratase (PDT).



Scheme 1.

T and P-protein alignment studies suggest that the CM activity of both proteins is located in the N-terminal region.[2] Indeed, subdomain cloning and expression studies on the P-protein have identified discrete separable mutase, dehydratase and regulatory domains.[3,4] However, the spatial relationship of active sites is not nearly so clearcut in the T-protein. Mutagenesis studies on the T-protein and kinetic studies using substrate analogues suggest that the CM and PDH reactions occur at overlapping[5] or perhaps closely proximal[6] active sites. The strongest evidence for two separate CM and PDH active sites comes from pH rate profile analysis,[7] and from various substrate and product-based inhibitors that affect the two catalytic activities with differing degrees of selectivity.[8] At one extreme, a well-known oxabicyclic mutase inhibitor[9] has been shown to inhibit CM in the T-protein without affecting PDH.[6]

In conjunction with our continuing interest in the mechanism and structure of PDH and other shikimate pathway enzymes, we report here the first example of an inhibitor, diacid **4** (Fig. 1), that disrupts PDH activity in the *E. coli* T-protein without affecting CM activity. Since the product of the CM reaction is the substrate of the PDH reaction, the competitive inhibition observed with **4** further strengthens the case for discrete binding pockets for the two enzymes.



4

Figure 1.

*Corresponding author. Tel.: +1-607-225-4174; fax: +1-607-255-6318; e-mail: bg18@cornell.edu

0960-894X/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00053-7

Our initial goal was to use known prephenate mimics as lead compounds around which to optimize an inhibitor specific for PDH. Reasoning that the prephenate binding site of PDH might resemble that in PDT, we decided to test PDH for inhibition by *S*-6,6′-dinitrobiphenic acid (*S*-DNBA), which had earlier been shown to inhibit PDT competitively as a prephenate mimic.[10] However, assays of *S*-DNBA showed no effect on PDH at 1 mM. Therefore, an array of known, low-molecular weight, racemic, mono-, di-, and tricarboxylic acids **4**–**28** (Fig. 2) was assembled and screened for PDH inhibition using a standard T-protein[11] assay [5 min, 37 °C, 50 mM Tris (pH 7.8), 2.5 mM EDTA, 20 mM mercaptoethanol, 0.01% BSA, and 333 μM chorismic acid ($K_m = 290$ μM)]. The residual activity of PDH was determined by monitoring the formation of NADH at 340 nm.[12]

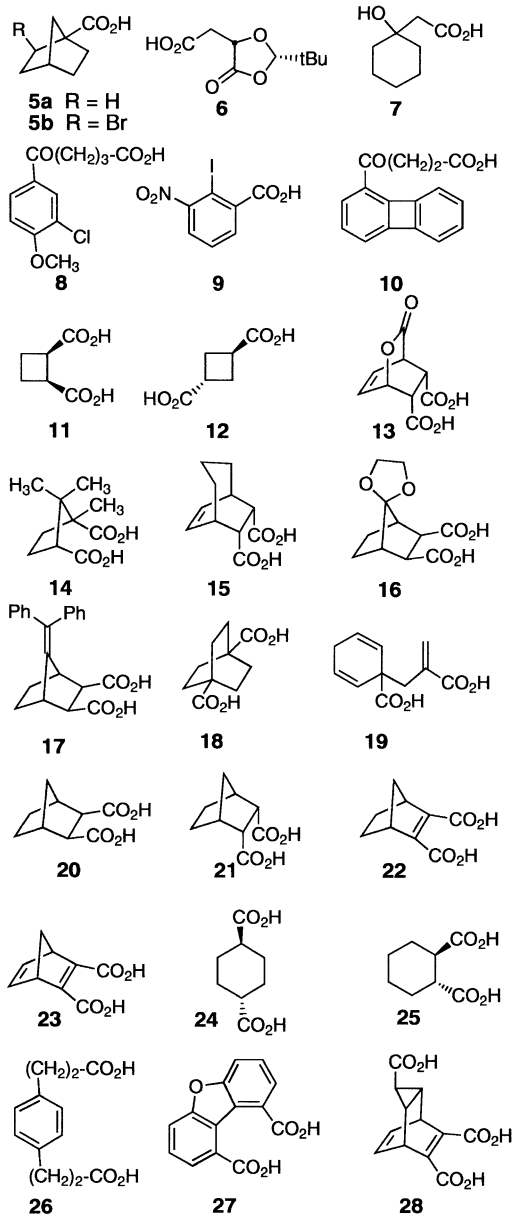


Figure 2.

None of the mono, di, or triacids **5**–**28** inhibited PDH at 1 mM concentrations in the initial screen. However, *trans*-2,3-pleiadanedicarboxylic acid (±)-**4**[13] displayed promising inhibition of PDH, with an IC_{50} value of 250 μM. From analysis of a Lineweaver–Burk plot (Fig. 3), inhibition of PDH by (±)-**4** was found to be competitive, with a K_I of 212 ± 10 μM. Moreover, (±)-**4** had no effect at concentrations up to 1 mM on the chorismate mutase activity of the T-protein.

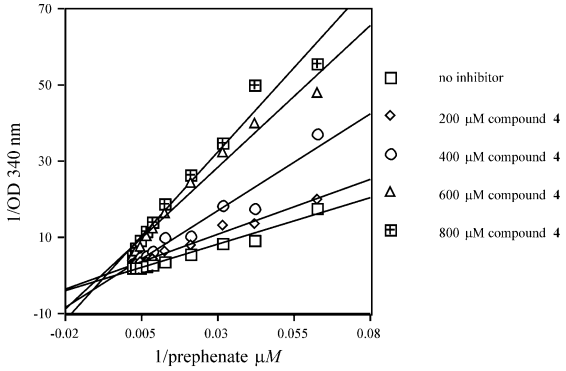


Figure 3. Reciprocal plot of the velocity of the PDH reaction versus the concentration of prephenate **2** in the presence and absence of diacid **4** (conditions: 40 mM Tris buffer, 0.4 mM EDTA, 80 mM mercaptoethanol, pH 7.5, 37 °C).

The promising selective activity of (±)-**4** prompted an investigation of close structural analogues. Attempts to epimerize diacid (±)-**4** to its *cis*-isomer were unsuccessful. An electronic substructure search uncovered the known, conformationally constrained analogues, monoacid **29**[14] and diacid **30** (Fig. 4).[15] However, neither compound inhibited CM or PDH in the T-protein.

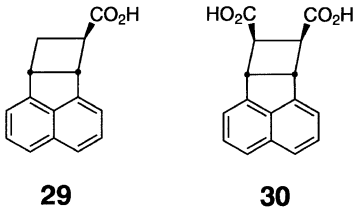


Figure 4.

In earlier studies using inhibitors to elucidate the spatial relationship between the mutase and dehydrogenase sites, Christopherson et al. observed that several chorismate and adamantane derivatives functioned as linear competitive inhibitors of the T-protein catalytic activities (Table 1).[16] Judging from the tabulated data, PDH inhibition by these substances (which were achiral or pure enantiomers) was comparable, or slightly superior, to that observed with (±)-**4**. However, only (±)-**4** selectively inhibited PDH in the T-protein.

In conclusion, we have identified an inhibitor of prephenate dehydrogenase that has no effect on the chorismate mutase activity of the *E. coli* T-protein. Our findings support the hypothesis that the CM and PDH catalytic domains may utilize discrete binding pockets within separate or partially overlapping active sites.

Table 1. Kinetic constants for known inhibitors of PDH and CM activities in the *E. coli* T-protein

Compd	K_I (CM) (μM)	K_I (PDH) (μM)
2-Hydroxyphenylacetate	320±40	170±30
Adamantane-1-COOH	430±40	310±140
Adamantane-1-acetate	220±60	130±30
Adamantane-1,3-diacetate	340±30	150±50
Adamantane-1-phosphonate	200±20	140±20
Chorismate-5,6-epoxide	230±30	130±20
5,6-Dihydro-5,6-dihydroxychorismate	410	110
Δ^6-Dihydrochorismate	430	590

Acknowledgements

We thank the National Institutes of Health (GM 24054, to B.G.) and the Department of Energy (DE-F G02–84ER13233, to D.B.W.) for financial support. We are also grateful to Professor J. Meinwald for providing samples of **29** and **30**. Support of the Cornell NMR Facility has been provided by NSF (CHE 7904825; PGM 8018643) and NIH (RR02002).

References and Notes

1. Haslam, E. In *Shikimic Acid Metabolism and Metabolites*; John Wiley & Sons: New York, 1993.
2. (a) Hudson, G. S.; Davidson, B. E. *J. Mol. Biol.* **1984**, *180*, 1023. (b) Hudson, G. S.; Wong, V.; Davidson, B. E. *Biochemistry* **1984**, *23*, 6240.
3. Pohnert, G.; Zhang, S.; Husain, A.; Wilson, D. B.; Ganem, B. *Biochemistry* **1999**, *38*, 12212.
4. Zhang, S.; Wilson, D. B.; Ganem, B. *Biochemistry* **2000**, *39*, 4722.
5. Heyde, E.; Morrison, J. F. *Biochemistry* **1978**, *17*, 1573.
6. Turnbull, J.; Morrison, J. F. *Biochemistry* **1990**, *29*, 10255.
7. Turnbull, J.; Cleland, W. W.; Morrison, J. F. *Biochemistry* **1991**, *30*, 7777.
8. Christofferson, R. I. *Int. J. Biochem. Cell Biol.* **1997**, *29*, 589.
9. Bartlett, P. A.; Nakagawa, Y.; Johnson, C. R.; Reich, S. H.; Luis, A. *J. Org. Chem.* **1988**, *53*, 3195.
10. Husain, A.; Galopin, C.; Zhang, S.; Pohnert, G.; Ganem, B. *J. Am. Chem. Soc.* **1999**, *121*, 2647.
11. The tyrA gene, which codes for the T-protein, was subcloned from the plasmid pKB 45, a derivative of pMB9 that contained a 6 kilobase segment of *E. coli* chromosomal DNA; Zurawski, G.; Brown, K.; Killingly, D.; Yanofsky, C. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4271.
12. Davidson, B. E.; Hudson, G. S. *Methods Enzymol.* **1987**, *142*, 440.
13. Prepared from 1,8-bis-(chloromethyl)naphthalene and tetra-(carboethoxy)ethane: Boeckelheide, V.; Vick, G. K. *J. Am. Chem. Soc.* **1956**, *78*, 653.
14. Petty, R. L.; Ikeda, M.; Samuelson, G. E.; Boriack, C. J.; Onan, K. D.; McPhail, A. T.; Meinwald, J. *J. Am. Chem. Soc.* **1978**, *100*, 2464.
15. Shields, J. E.; Gavrilovic, D.; Kopecky, J.; Hartmann, W.; Heine, H.-G. *J. Org. Chem.* **1974**, *39*, 515.
16. Christopherson, R. I.; Heyde, E.; Morrison, J. F. *Biochemistry* **1983**, *22*, 1650.