after purification by flash chromatography (200 g silica gel, 35% ethyl acetate/hexane, 40-mL fractions), 3.1 g (97%) of **68**. Anal. ($C_{17}H_{24}N_2O_4$) C, H, N.

Methyl 5-(Cyanomethyl)-1-(*tert*-butoxycarbonyl)-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylate (70). As for **29**, hydrogenation of 3.1 g (9.6 mmol) of **68** in 95 mL of ethanol with 1.0 g of 5% Pd/C at room temperature at 60 psi for 1 h gave, after purification by flash chromatography (120 g silica gel, 35% ethyl acetate/hexane, 100-mL fractions), 2.2 g (73%) of **70** as a mixture of diastereomers at C_5. Anal. ($C_{17}H_{26}N_2O_4$) C, H, N.

5-(1*H*-Tetrazol-5-ylmethyl)-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylic Acid (72). As for **32a**, 2.1 g (6.6 mmol) of **70** and 4.4 g (13.1 mmol) of azidotri-*n*-butylstannane gave 1.4 g (86%) of **72** as a mixture of diastereomers at C_5: mp 154-157 °C; ^{1}H NMR (D_2O) δ 4.12 (m, 1 H), 3.72 (m, 1 H), 2.82 (m, 2 H), 1.40-2.50 (m, 8 H), 1.12 (m, 1 H), 0.75 (m, 1 H). Anal. ($C_{11}H_{17}N_5O_2 \cdot 0.5H_2O$) C, H, N.

Acknowledgment. The authors would like to thank Ron Lawson and Charles C. Hillman, Jr. for their technical assistance and the Physical Chemistry Department of Lilly Research Laboratories for spectral data and elemental analyses.

Carboxamide Group Conformation in the Nicotinamide and Thiazole-4-carboxamide Rings: Implications for Enzyme Binding†

Hong Li and Barry M. Goldstein*

Department of Biophysics, University of Rochester Medical Center, Rochester, New York 14642. *Received March 16, 1992*

Ab initio computations (RHF/6-31G*//3-21G*) were performed on the thiazole-4-carboxamide group found in the antitumor drug tiazofurin and its dehydrogenase-binding anabolite thiazole-4-carboxamide adenine dinucleotide (TAD). Results indicate that the carboxamide group is constrained in the conformation in which the amino group is cis-planar to the ring nitrogen. This finding is consistent with carboxamide conformations observed in crystal structures of the thiazole nucleosides. In contrast, ab initio computations on the nicotinamide and dihydronicotinamide rings found in the cofactors NAD^+ and NADH indicate two stable conformations for the carboxamide group. This finding confirms previous computational studies and is consistent with results from a survey of the Cambridge Structural Database. Natural bond orbital analysis indicates that the low-energy carboxamide conformers of all three heterocycles are stabilized by a combination of electrostatic and charge transfer interactions. A survey of the Protein Data Bank indicates that the carboxamide group conformation in TAD is constrained to that favored by dehydrogenase-bound NAD(P)(H).

Introduction

The thiazole nucleoside tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, NSC 286193) has demonstrated clinically effective antitumor activity.[1,2] Recently, attention has also focused on tiazofurin's ability to induce differentiation in neoplastic cells[3-6] and to inhibit G-protein-mediated signaling mechanisms.[6-9] Each of these biological effects appears related to a shutdown of guanine nucleotide synthesis[1-5,10] produced by a dinucleotide anabolite of tiazofurin called tiazofurin adenine dinucleotide (TAD, Figure 1a).[11-13] TAD is a neutral analogue of the cofactor nicotinamide adenine dinucleotide (NAD^+) in which the nicotinamide ring is replaced by the thiazole-4-carboxamide group (Figure 1b).[11-13] TAD acts as an inhibitor of inosine monophosphate dehydrogenase (IMPd), the NAD^+-dependent enzyme catalyzing the rate-limiting step in guanine nucleotide synthesis.[14] Kinetic and modeling studies suggest that TAD inhibits IMPd by mimicking NAD binding at the cofactor site.[15,16]

Although there is no crystal structure of an IMPd-TAD complex, the structural features of free tiazofurin have been studied extensively by X-ray crystallography[17] and quantum chemical computations.[18] X-ray crystallographic studies of a series of thiazole nucleosides[17-21] suggest that the TAD molecule possesses two unique conformational restrictions compared with NAD^+ and NADH. Rotation about both the C-glycosidic bond (C1'-C2) and about the bond to the carboxamide group (C4-C6) is constrained

* Author to whom correspondence should be addressed.

† Abbreviations: TAD: thiazole-4-carboxamide adenine dinucleotide; IMPd: inosine monophosphate dehydrogenase; NAD^+: nicotinamide adenine dinucleotide; NADH: dihydronicotinamide adenine dinucleotide; NADP: nicotinamide adenine dinucleotide phosphate; NADPH: dihydronicotinamide adenine dinucleotide phosphate; NAD(P)(H): NAD^+ or NADH or $NADP^+$ or NADPH; NBO: natural bond orbital.

(1) Tricot, G. J.; Jayaram, H. N.; Lapis, E.; Natsumeda, Y.; Nichols, C. R.; Kneebone, P.; Heerema, N.; Weber, G.; Hoffman, R. Biochemically Directed Therapy of Leukemia with Tiazofurin, a Selective Blocker of Inosine 5'-phosphate Dehydrogenase Activity. *Cancer Res.* **1989**, *49*, 3696-3701.

(2) Tricot, G. J.; Jayaram, H. N.; Nichols, C. R.; Pennington, K.; Lapis, E.; Weber, G.; Hoffman, R. Hematological and Biochemical Action of Tiazofurin (NSC 286193) in a Case of Refractory Acute Myeloid Leukemia. *Cancer Res.* **1987**, *47*, 4988-4991.

(3) Goldstein, B. M.; Leary, J. F.; Farley, B. A.; Marquez, V. E.; Levy, P. C.; Rowley, P. T. Induction of HL60 Cell Differentiation by Tiazofurin and its Analogues: Characterization and Efficacy. *Blood* **1991**, *78*, 593-598.

(4) Kiguchi, K.; Collart, F. R.; Henning-Chubb, C.; Huberman E. Cell Differentiation and Altered IMP Dehydrogenase Expression Induced in Human T-lymphoblastoid Leukemia Cells by Mycophenolic Acid and Tiazofurin. *Exp. Cell Res.* **1990**, *187*, 47-53.

(5) Collart, F. R.; Huberman, E. Expression of IMP Dehydrogenase in Differentiating HL-60 cells. *Blood* **1990**, *75*, 570-576.

(6) Olah, E.; Natsumeda, Y.; Ikegami, T.; Kote, Z.; Horanyi, M.; Szelenyi, J.; Paulik, E.; Kremmer, T.; Hollan, S. R.; Sugar, J.; Weber, G. Induction of Erythroid Differentiation and Modulation of Gene Expression in K-562 Leukemia Cells. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6533-6537.

(7) Parandoosh, Z.; Rubalcava, B.; Matsumoto, S. S.; Jolley, W. B.; Robins, R. K. Changes in Diacylglycerol and Cyclic GMP During the Differentiation of Human Leukemia K562 Cells. *Life Sci.* **1990**, *46*, 315-320.

(8) Parandoosh, Z.; Robins, R. K.; Belei, M.; Rubalcava, B. Tiazofurin and Selenazofurin Induce Depression of cGMP and Phosphatidylinositol Pathway in L1210 Leukemia Cells. *Biochem. Biophys. Res. Commun.* **1989**, *164*, 869-874.

0022-2623/92/1835-3560$03.00/0

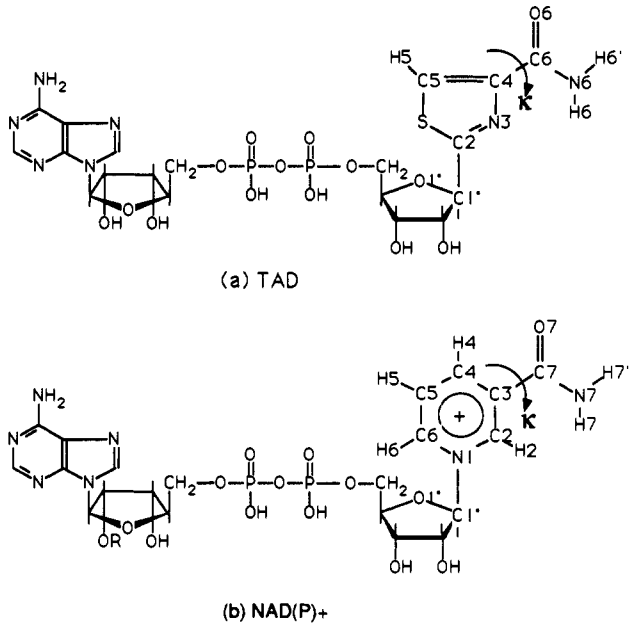

Figure 1. TAD (a) and NAD(P)(H) (b) structural formula illustrating atomic numbering. R = H for NAD^+, R = PO_3H_3 for $NADP^+$. The reduced species NADH and NADPH are obtained by protonating the C4 carbon.

(Figure 1).[18] Values of the C-glycosidic bond torsion angle (χ = S–C2–C1′–O1′) observed in all structures of thiazole nucleosides are under 60°.[19] Those for the carboxamide torsion angle (κ = O6–C6–C4–C5) are primarily within the range −15° to +10°.[18,19-21] Quantum chemical computations have confirmed that the restriction on C-glycosidic bond rotation is imposed by an intramolecular electrostatic interaction between the heteroatom sulfur and the ribose oxygen.[18] In the present work we show that only one conformation (κ = 0°) is stable for the carboxamide group of the thiazole-4-carboxamide ring.

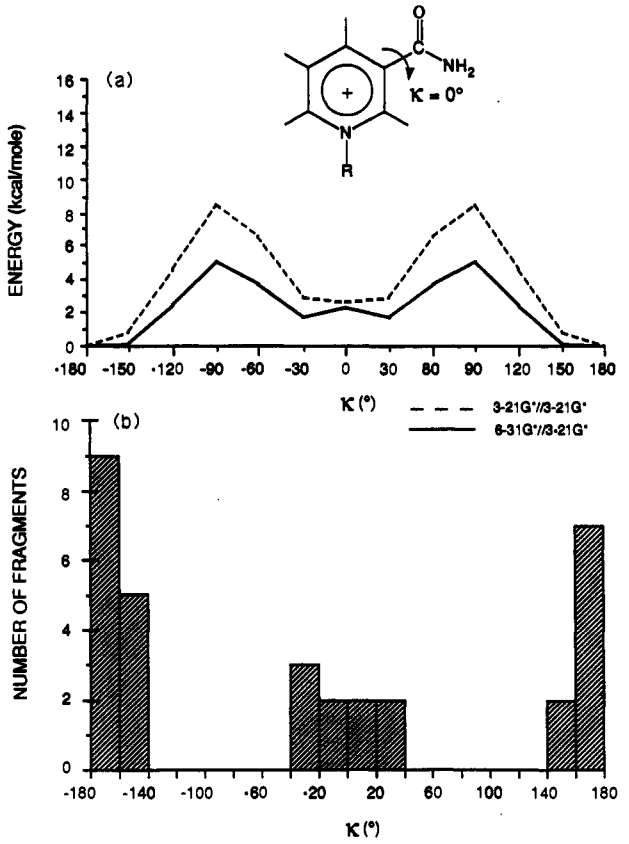

Figure 2. (a) Energy profile for the carboxamide dihedral angle κ in the nicotinamide ring. Each point was obtained by fixing κ at the indicated value and computing the energy for the fully optimized fragment at the 6-31G*//3-21G* and 3-21G*//3-21G* levels. The global minimum is normalized to 0 kcal/mol. The fragment minimized is illustrated, with R = H. (b) Histogram of carboxamide dihedral angles observed in crystal structures of the nicotinamide ring obtained from a search of the Cambridge Structural Database. The fragment used in the search is illustrated in (a), with R = any substituent.

The binding of $NAD(P)^+$ and NAD(P)H to dehydrogenases or reductases is required for the catalytic function of these enzymes. The carboxamide group of $NAD(P)^+$ and NAD(P)H plays an important role in the specific orientation of the nicotinamide ring relative to bound substrate and enzyme catalytic groups.[22] Given

(9) Kharbanda, S. M.; Sherman, M. L.; Kufe, D. W. Effects of Tiazofurin on Guanine Nucleotide Binding Regulatory Proteins in HL-60 Cells. *Blood* **1990**, *75*, 583–588.

(10) Sokoloski, J. A.; Blair, O. C.; Sartorelli, A. C. Alterations in Glycoprotein Synthesis and Guanosine Triphosphate Levels Associated with the Differentiation of HL-60 Leukemia Cells Produced by Inhibitors of Inosine 5′-phosphate Dehydrogenase. *Cancer Res.* **1986**, *46*, 2314–2319.

(11) Cooney, D. A.; Jayaram, H. N.; Gebeyehu, G.; Betts, C. R.; Kelly, J. A.; Marquez, V. E.; Johns, D. G. The Conversion of 2-β-D-ribofuranosylthiazole-4-carboxamide to an Analogue of NAD with Potent IMP Dehydrogenase-inhibitory Properties. *Biochem. Pharmacol.* **1982**, *31*, 2133–2136.

(12) Kuttan, R.; Robins, R. K.; Saunders, P. P. Inhibition of Inosinate Dehydrogenase by Metabolites of 2-β-D-ribofuranosylthiazole-4-carboxamide. *Biochem. Biophys. Res. Commun.* **1982**, *107*, 862–868.

(13) Cooney, D. A.; Jayaram, H. N.; Glazer, R. I.; Kelly, J. A.; Marquez, V. E.; Gebeyehu, G.; van Coot, A. C.; Zwelling, L. A.; Johns, D. G. Studies on the Mechanism of Action of Tiazofurin Metabolism to an Analog of NAD with Potent IMP Dehydrogenase-Inhibitory Activity. *Adv. Enzyme Regul.* **1983**, *21*, 271–303.

(14) Jackson, R. C.; Weber, G.; Morris, H. P. IMP Dehydrogenase - An Enzyme Linked with Proliferation and Malignancy. *Nature* **1975**, *256*, 331–333.

(15) Goldstein, B. M.; Bell, J. E.; Marquez, V. E. Dehydrogenase Binding by Tiazofurin Anabolites. *J. Med. Chem.* **1990**, *33*, 1123–1127.

(16) Hedstrom, L.; Wang, C. C. Mycophenolic Acid and Thiazole Adenine Dinucleotide Inhibition of *Tritrichomonas foetus* Inosine 5′-monophosphate Dehydrogenase: Implications on Enzyme Mechanism. *Biochemistry* **1990**, *29*, 849–854.

(17) Goldstein, B. M.; Takusagawa, F.; Berman, H. M.; Srivastava, P.; Robins, R. K. Structural Studies of a New Antitumor Agent: Tiazofurin and its Inactive Analogues. *J. Am. Chem. Soc.* **1983**, *105*, 7416–7422.

(18) Burling, F. T.; Goldstein, B. M. Computational Studies of Nonbonded Sulfur–Oxygen and Selenium–Oxygen Interactions in the Thiazole and Selenazole Nucleosides. *J. Am. Chem. Soc.* **1992**, *114*, 2313–2320.

(19) Goldstein, B. M.; Mao, D. T.; Marquez, V. E. Ara-tiazofurin: Conservation of Structural Features in an Unusual Thiazole Nucleoside. *J. Med. Chem.* **1988**, *31*, 1026–1031.

(20) Burling, F. T.; Gabrielsen, B.; Goldstein, B. M. Structures of the 2′,3′-Dideoxy and 2′,3′-Didehydro-2′,3′-dideoxy Analogs of Tiazofurin. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1991**, *C47*, 1272–1275.

(21) Burling, F. T.; Hallows, W. A.; Phelan, M. J.; Gabrielsen, B.; Goldstein, B. M. Structures of the 4-Cyano and 4-Methylamidate Analogs of Tiazofurin. *Acta Crystallogr., Sect. B: Struct. Sci.*, in press.

(22) (a) Grau, U. In *The Pyridine Nucleotide Coenzymes*; Everse, J., Anderson, B., You, K.-S., Eds.; Academic Press: New York, 1982; Chapt. 5. (b) Eklund, H.; Brändén, C.-I. In *Pyridine Nucleotide Coenzymes. Chemical, Biochemical, and Medical Aspects.*; Dolphin, D., Avramovic, O., Poulson, R., Eds.; John Wiley and Sons: New York, 1987; Vol. II, Part A, Chapt. 4.

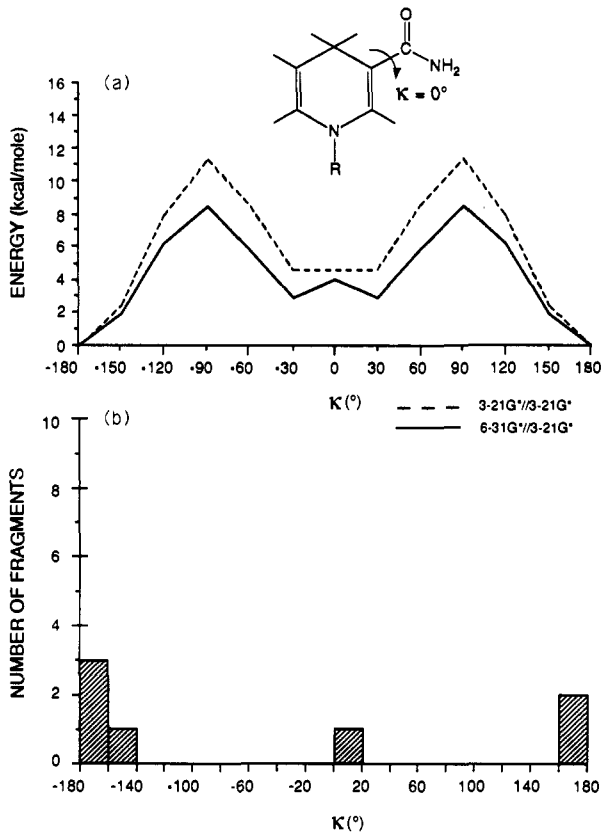

Figure 3. (a) Energy profile for the carboxamide dihedral angle κ in the dihydronicotinamide ring. Each point was obtained by fixing κ at the indicated value and computing the energy for the fully optimized fragment at the 6-31G*//3-21G* and 3-21G*//3-21G* levels. The global minimum is normalized to 0 kcal/mol. The fragment minimized is illustrated, with R = H. (b) Histogram of carboxamide dihedral angles observed in crystal structures of the dihydronicotinamide ring obtained from a search of the Cambridge Structural Database. The fragment used in the search is illustrated in (a), with R = any substituent.

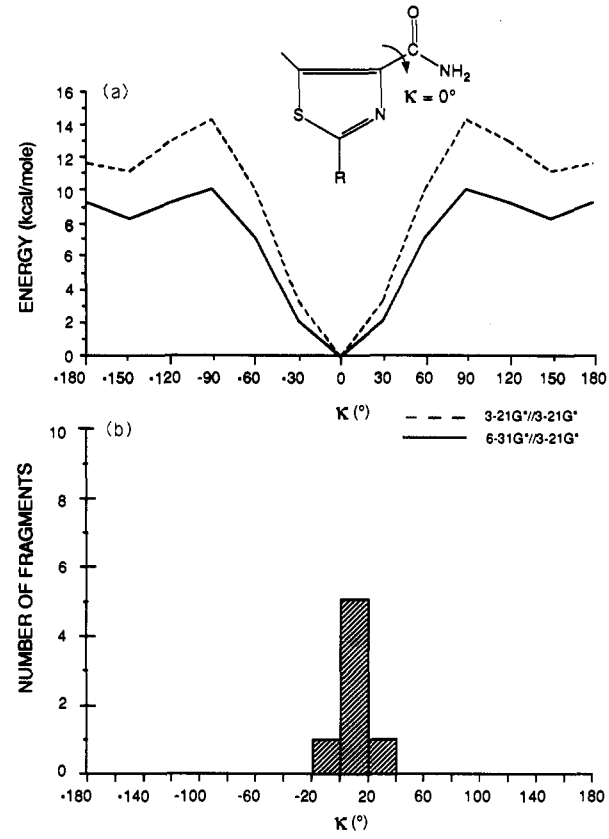

Figure 4. (a) Energy profile for the carboxamide dihedral angle κ in the thiazole-4-carboxamide ring. Each point was obtained by fixing κ at the indicated value and computing the energy for the fully optimized fragment at the 6-31G*//3-21G* and 3-21G*//3-21G* levels. The global minimum is normalized to 0 kcal/mol. The fragment minimized is illustrated, with R = H. (b) Histogram of carboxamide dihedral angles observed in crystal structures of the thiazole-4-carboxamide ring obtained from a search of the Cambridge Structural Database. The fragment used in the search is illustrated in (a), with R = any substituent.

that dehydrogenase binding by the active anabolite TAD occurs at the cofactor site, it is of interest to know (1) how a constraint on carboxamide rotation in TAD compares with that observed in the regular cofactor and (2) how this constraint may influence the ability of TAD to mimic cofactor binding in dehydrogenases. Thus, we also examine carboxamide group conformation on reduced and oxidized nicotinamide rings in both free and enzyme-bound structures.

We present here ab initio calculations of energy as a function of carboxamide torsion angle for the thiazole-4-carboxamide, nicotinamide, and dihydronicotinamide groups at the 6-31G*//3-21G* level.[23] These results are compared with those from X-ray crystallographic structures of the free and enzyme-bound groups, as obtained from database surveys. Finally, natural bond orbital (NBO) analysis[24] is applied in order to interpret these results within the conceptual framework of covalent and noncovalent interactions. This combination of computational and crystallographic data is used to infer the preferred conformations of the carboxamide groups in TAD, $NAD(P)^+$, and NAD(P)H in solution and enzyme-bound.

Findings suggest that the carboxamide group in TAD is constrained in the position favored for dehydrogenase binding.

Methods

Ab initio molecular orbital calculations were carried out, using the Gaussian90 program,[25] on the model fragments shown in Figures 2a, 3a, and 4a. There is some confusion in the literature regarding the description of the conformation of the carboxamide group relative to the pyridinium ring in the nicotinamide moiety.[26-30] In this study,

(23) Hehre, J. W., Radom, L., Scheleyer, P. V. R., Pople, J. A. *Ab Initio Molecular Orbital Theory*; John Wiley-Interscience Publication: New York, 1986; Chapt. 4.
(24) Reed, A. E.; Curtiss, L. A.; Weinhold, F. Intermolecular Interactions from a Natural Bond Orbital, Donor-Acceptor Viewpoint. *Chem. Rev.* **1988**, *88*, 889–926.
(25) Frisch, M. J.; Head-Gordon, M.; Schlegel, H. B.; Raghavachari, K.; Brinkley, J. S.; Gonzalez, C.; Defrees, D. J.; Fox, D. J.; R. A.; Whiteside, R. A.; Seeger, R.; Melius, C. F.; Baker, J.; Kahn, L. R.; Stewart, J. J. P.; Fluder, E. M.; Topiol, S.; Pople, J. A. *Gaussian 88*; Gaussian, Inc., Pittsburgh, PA 15213.
(26) Cummins, P. L.; Gready, J. E. Mechanistic Aspects of Biological Redox Reactions Involving NADH 1: *Ab Initio* Quantum Chemical Structures of the 1-Methyl-Nicotinamide and 1-Methyl-Dihydronicotinamide Coenzyme Analogues. *J. Mol. Struct.: THEOCHEM* **1989**, *183*, 161–174.
(27) Bossaerts, J.; Alderweireldt, F. C.; Geerlings, P. *Ab Initio* Quantum-Chemical Study on the Influence of Methyl Substitution on the Redox Behaviour of 1,4-Dihydronicotinamides. Part 1: Unperturbed Molecules and Perturbations towards a Possible Transition State. *Heterocycles* **1986**, *24*, 2519–2538.
(28) Bohm, S.; Kuthan, J., An *ab initio* MO Study of Conformation Structure of Nicotinamide and Its Derivatives. *Collect. Czech. Chem. Commun.* **1983**, *48*, 1842–1853.

the torsion angle κ was used, defined by atoms O7–C7–C3–C4 (Figure 1b). The conformation for which $\kappa = 0°$ is illustrated in Figure 1b and is referred to here as "cis-planar". The conformation for which $\kappa = \pm 180°$ is described as "trans-planar", the terms cis and trans referring here to the relative orientations of O7 and C4. The same terms are used for the thiazole-4-carboxamide moiety, with the exception that κ is defined by atoms O6–C6–C4–C5 (Figure 1a).

Using these conventions, rotational energy profiles were computed as follows. Potential energies were obtained every 30° starting from $\kappa = -180°$ and ending at $\kappa = 0°$ by fully optimizing a fragment when fixing κ at the desired value. The optimization for the ith κ angle was carried out using geometries and wave functions as initial guesses from checkpoint files of optimized fragments at the $(i-1)$th κ angle. It has been shown that a polarization-added basis set is sufficient for rotational barrier calculations in most cases.[23] Furthermore, electron correlation has little effect on rotational barriers.[23] Therefore, there was no consideration of electron correlation effects in these computations. 3-21G* was used as the basis set for the geometry optimization and 6-31G* was used for single-point SCF energy values at each κ point. Thus, each point represents a calculation at the RHF 6-31G*//RHF 3-21G* level. The computed results are plotted in Figures 2a, 3a, and 4a, respectively, for NAD(P)$^+$, NAD(P)H, and TAD model fragments. Results at the lower RHF 3-21G*//RHF 3-21G* level are also plotted for comparison with earlier studies. All curves are on the same scale and are normalized to 0 kcal/mol at the global minimum. Notice the symmetry with respect to the ring plane in each fragment, i.e. negative κ values are equivalent to positive ones. In Figures 2a, 3a, and 4a, energy profiles are symmetrized about $\kappa = 0°$. Profiles are presented from $\kappa = -180°$ to $\kappa = 180°$ for comparison with results of the database surveys (see below).

It is important to recognize that these in vacuo computations ignore solvent–solute intermolecular interactions. Thus, although the calculated rotational barriers have been carried out at a high level of theory, numerical values should be treated as upper bounds for actual barrier heights in solution.[24]

In order to rationalize chemically the observed barriers to rotation of the carboxamide groups with respect to the base rings (Figures 2a, 3a, 4a), we performed detailed natural bond orbital (NBO) analyses on these model fragments using the NBO program incorporated in Gaussian90.[31] NBO analysis allows one to isolate interactive energies due to electron density delocalization, or charge transfer, and to relate these interactions to either H-bonding or other types of nonbonded interactions based on bond orbital interaction concepts.[24] NBO analysis decomposes the internal energy of a chemical complex, E,

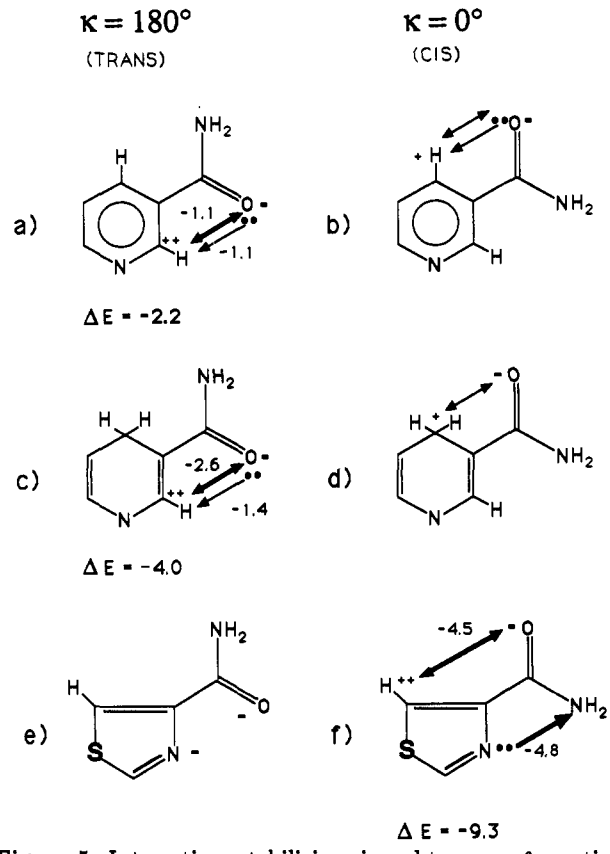

Figure 5. Interactions stabilizing cis and trans conformations of the nicotinamide (a,b), dihydronicotinamide (c,d) and thiazole-4-carboxamide (e,f) rings. Filled circles represent lone-pair orbitals and "+" and "–" signs indicate regions of positive and negative charge as determined by population analyses. Double-headed arrows indicate major electrostatic interactions. Single-headed arrows indicate major charge transfer interactions in the direction from donor to acceptor. The thickness of the arrow indicates the relative magnitude of the interaction. The number above and below each arrow is the difference in total charge transfer energy (ΔE_{CT}, above) and non charge transfer energy (ΔE_{NCT}, below) stabilizing the low-energy conformer. ΔE is the total energy difference between the cis and trans conformers, and is listed under the lower energy conformer.

into a charge transfer energy, E_{CT}, and a non-charge transfer energy, E_{NCT}, the latter including electrostatic and exclusion–repulsion terms. Thus $E = E_{CT} + E_{NCT}$. Although this method of energy decomposition is neither unique nor required by theory, it has the advantage of interpreting the computational results in terms of classical chemical concepts.[24]

The value of E_{CT} between the heterocycle and the carboxamide group was obtained for each of the three fragments by computing the difference in SCF energy before and after deletion of the Fock matrix elements describing all delocalized interactions between the carboxamide group and the heterocycle.[24,31] Values of the total energy E, charge transfer energy E_{CT}, and non-charge transfer energy E_{NCT} were compared for the cis and the trans conformations of each fragment. The differences in these values (i.e. $\Delta E = E(\text{cis}) - E(\text{trans})$) are given in Figure 5. To further pinpoint the important delocalization interactions, energies were obtained following deletion of Fock matrix elements between selected donor–accept type orbitals.[24,31] NBO studies were performed using both the 3-21G* and 6-31G* wave functions on fragment geometries optimized at the 3-21G* level. These results are illustrated schematically in Figure 5.

Computational results were also compared with exper-

(29) Donkersloot, M. C. A.; Buck, H. M. The Hydride-Donation Reaction of Reduced Nicotinamide Adenine Dinucleotide. 2. MINDO/3 and STO-3G Calculations on the Role of the CONH_2 Group in Enzymatic Reactions. *J. Am. Chem. Soc.* 1981, *103*, 6554–6558.

(30) Perahia, D.; Pullman, B.; Saran, A. A Molecular Orbital Probe into the Conformation of Pyridine Coenzymes and Their Constituents. In *Structure and Conformation of Nucleic Acids and Protein-Nucleic Acid Interactions*; Sundaralingam, M., Rao, S. T., Eds.; University Park Press: Baltimore, MD, 1975; pp 685–708.

(31) Glendening, E. D.; Reed, A. E.; Carpenter, J. E.; Weinhold, F. NBO 3.0 program manual. Theoretical Chemistry Institute and Department of Chemistry, University of Wisconsin, Madison, WI, 1990.

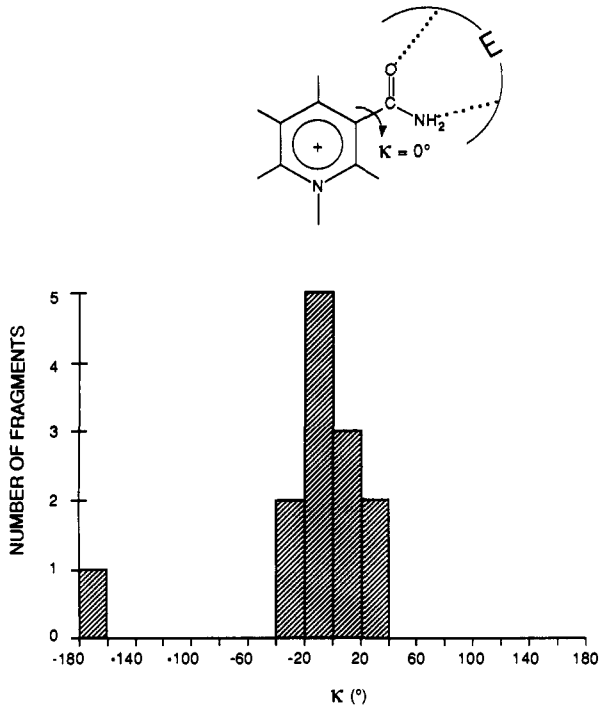

Figure 6. Histogram of carboxamide dihedral angles observed in crystal structures of enzyme-bound NAD(P)(H) obtained from a search of the Brookhaven Protein Data Bank (Table I). The insert schematically illustrates hydrogen bonding between the enzyme and the carboxamide group of the bound cofactor $(NAD(P)^+$ is shown). The two lactate dehydrogenase structures from dogfish (Table I) are represented by only one value of κ.

imentally obtained carboxamide group conformations in unbound molecules. A search of the Cambridge Structure Database (Version 4.5)[32] was carried out for all crystal structures containing thiazole-4-carboxamide, nicotinamide, and dihydronicotinamide moieties. Fragments used in the search are shown in Figures 2a, 3a, and 4a. The "constrain-structures" option in the QUEST routine was used to constrain the number of hydrogens on each heavy atom. Geometries of all fragments, as well as the oxidation states of the nitrogen-containing heterocycles, were checked with the original literature where possible.[33] Values of the carboxamide dihedral angle κ for each fragment were obtained using the GSTAT88 program. Figures 2b, 3b, and 4b show the distribution of κ observed for the structures containing nicotinamide, dihydronicotinamide, and thiazole-4-carboxamide groups, respectively. These histograms are compared with the computed energy profiles illustrated in Figures 2a, 3a, and 4a. Each histogram is on the same scale. Values of κ are reported in the range $-180°$ to $180°$, as they appear in the database. This results from asymmetry in both the intra- and intermolecular environment of the rings.

In order to determine the carboxamide conformations in protein-bound cofactors, a search of the Brookhaven Protein Data bank[34] (January, 1992) was carried out for all binary and ternary complexes containing NAD(H) or NADP(H). For each structure, the enzyme-bound cofactor was displayed on a graphics system and the value of κ for the carboxamide group obtained. Coordinates were obtained from all entries containing bound cofactor. Twelve entries containing twenty crystallographically independent protein–cofactor complexes were examined. Information for each structure is listed in Table I.[35] The distribution of κ values for each enzyme-bound cofactor is plotted in Figure 6.

Results

The Carboxamide Group of the Nicotinamide and Dihydronicotinamide Rings. Figure 2a shows the energy as a function of carboxamide group rotation in the nicotinamide ring at both the 3-21G*//3-21G* and 6-31G*//3-21G* levels. Qualitative features of both curves are similar. The optimized $NAD(P)^+$ model fragment shows a global minimum at $\kappa = \pm 180°$, i.e. the conformation in which the carboxamide oxygen O7 is trans-planar to the pyridinium carbon C4. A second broad minimum is observed about $\kappa = 0° \pm 30°$, the cis-planar conformation. The 6-31G*//3-21G* calculation shows a local minima at $\kappa = \pm 30°$ which is $\sim$0.5 kcal/mol lower than the energy at $\kappa = 0°$. In either case, the range of conformers between $\kappa = -30°$ and $\kappa = 30°$ is clearly preferred over all but the trans-planar conformation ($\kappa = \pm 180°$), which remains at the global minimum. The energy difference between the cis and trans conformers is about 2 kcal/mol using the 6-31G*//3-21G* basis set, with $\kappa = \pm 180°$ the preferred dihedral angle. The energy barrier between these two conformations is about 5 kcal/mol (Figure 2a).

These results were compared with the distribution of carboxamide dihedral angles observed in crystal structures of compounds containing the nicotinamide ring (Figure 2b).[33] The carboxamide angles in these nicotinamide structures are distributed around two distinct values, $\kappa = 0°$ and $\kappa = \pm 180°$. The number of conformers with $\kappa = \pm 180°$ is significantly higher than those with $\kappa = 0°$ (Figure 2b).

X-ray crystallographic structures provide "snap shots" of molecular conformations in the crystallization solution. Although the conformation of a molecule in the crystal environment is influenced by both inter- and intramolecular interactions, the latter tend to be systematic.[32] Therefore, the distribution of conformations observed in a series of related crystal structures reflects primarily intramolecular interactions, and is the distribution expected to occur in solution.[32] The larger the number of fragments observed at a given conformation in the crystal state, the lower the relative energy of that conformation and the greater its population in solution. Thus, the results shown in Figure 2b support the computational results shown in Figure 2a. These findings suggest that, in solution, the nicotinamide ring adopts two major conformer populations. These distribute the carboxamide group about the cis- and trans-planar conformations, the trans-planar conformer being preferred.

Figures 3a and 3b show analogous computational and crystallographic findings for the dihydronicotinamide ring. Results are qualitatively similar to those for the nicotinamide ring. Reduction of the ring does raise the energy of the cis-planar conformation ($\kappa = 0°$) by $\sim$0.5 kcal/mol relative to the minima at $\kappa = \pm 30°$ for the 6-31G*//3-21G* calculation. Nevertheless, the conformers between $\kappa = -30°$ and $\kappa = 30°$ remain at local minima and the trans-planar conformation ($\kappa = \pm 180°$) remains at the global

(32) Allen, F. H.; Kennard, O.; Taylor, R. Systematic Analysis of Structural Data as a Research Technique in Organic Chemistry. *Acc. Chem. Res.* **1983**, *16*, 146–153 and references therein.

(33) Citations for all structures retrieved from the Cambridge Structural Database are listed in supplementary Table I.

(34) Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer, E. F.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanoudhi, T.; Tasumi, M. The Protein Data Bank: A Computer-based Archival File for Macromolecular Structures. *J. Mol. Biol.* **1977**, *112*, 535–542.

(35) Citations for all structures retrieved from the Brookhaven Protein Data Bank are listed in supplementary Table II.

minimum. Results from the database survey, although limited, are consistent (Figure 3b).[33]

Several computational studies of carboxamide rotation in the nicotinamide and dihydronicotinamide moieties have appeared.[26-30] In general, these have been carried out using semiempirical or low-level ab initio methods. An exception is the thorough investigation of 1-methylnicotinamide and 1-methyldihydronicotinamide by Cummins and Gready.[26] This work presents computations for carboxamide conformations at the 6-31G//3-21G level. Unlike the present study, this work did not employ an extended basis set, and both the heterocycles and the carboxamide groups were constrained to be planar during the geometry optimizations. However, results for carboxamide conformations in both the nicotinamide and dihydronicotinamide moieties are in qualitative agreement with those presented here: a global minimum at $\kappa = \pm 180°$ with a local minimum about $\kappa = 0°$.

Results presented above clearly indicate that the trans conformation of the carboxamide group is more stable than the cis conformation in both the reduced and oxidized nicotinamide rings. In order to gain insight into the type of interactions stabilizing the trans conformer, computational results were subjected to natural bond order (NBO) analysis.[24] In this case, NBO analysis allows one to decompose the total interaction energy, E, between the carboxamide group and the heterocyclic ring, into a charge transfer energy, E_{CT} and a non-charge transfer energy, E_{NCT}. The charge transfer component is explained in terms of the donation of electron density, usually by a bonding or lone-pair orbital, to an antibonding orbital. If the latter involves a hydrogen atom, this interaction can be viewed as an H-bond.[24] The non-charge transfer energy, E_{NCT}, is the component of the interaction energy due to electrostatic and exclusion–repulsion effects.

Results of the NBO analysis are summarized schematically in Figure 5. For the oxidized and reduced nicotinamide rings, the energy differences between the more stable trans conformer and the cis conformer ($\Delta E = E$(cis) $- E$(trans)) are listed. The major interactions stabilizing both cis and trans conformations are also illustrated. Single-headed arrows represent charge transfer interactions from donor to acceptor orbitals in the direction of the arrow. Double-headed arrows represent non-charge transfer interactions, primarily electrostatic in origin. Numbers adjacent to the arrows indicate the amount by which this component of the total energy stabilizes the lower energy conformer. For example, −1.1 of the total −2.2 kcal/mol stabilization of the lower energy trans conformer of the nicotinamide ring (Figure 5a) comes from several charge transfer interactions. These primarily involve donation of charge by one lone-pair orbital on the carbonyl oxygen (illustrated in figure 5 by the filled dots) to various antibonding orbitals on the heterocycle. These include a hydrogen bond-like interaction between this carbonyl oxygen lone pair and a σ^*(C2–H2) antibonding orbital. Electrostatic interactions contribute the remaining −1.1 kcal/mol stabilization for the trans conformer of the nicotinamide ring. Again, this involves primarily interactions between the net negatively charged carbonyl oxygen (−0.62e) and the strongly positively charged atoms C2 and H2 (+0.44e total). Recall from Figure 2 that the cis conformer is also at an energy minimum, albeit at an energy 2.2 kcal/mol higher than that of the trans conformer. This conformer (Figure 5b) is also stabilized by an O(lone pair) $\rightarrow \sigma^*$(C4–H4) hydrogen bond-like interaction, although other charge transfer interactions are reduced (not illustrated). The electrostatic interactions between the carbonyl oxygen and the heterocycle are also maintained. However, there is significantly less positive charge in the region of C4 than in the region of C2, the total net positive charge on C4 and H4 being only +0.30e. Thus, electrostatic stabilization of this conformer is also significantly reduced.

Results for the dihydronicotinamide ring are similar. However, the favorable O(lone pair) $\rightarrow \sigma^*$(C4–H4) interaction seen in the cis conformer of the nicotinamide ring (Figure 5b) is lost in the cis conformer of the dihydronicotinamide ring (Figure 5d). Thus, both electrostatic and charge transfer stabilizations of this conformer are reduced relative to the trans conformer of the reduced ring (Figure 5c). The energy of the cis conformer is thus increased relative to the trans conformer, creating the larger 4.0 kcal/mol difference between the two seen in the dihydronicotinamide moiety.

The Carboxamide Group of the Thiazole-4-carboxamide Moiety. We now turn our attention to the thiazole-4-carboxamide moiety, found in both the parent compound tiazofurin and the dehydrogenase-binding anabolite TAD. The computed energy profiles at both the 3-21G* and 6-31G* levels for the thiazole-4-carboxamide ring show only one minimum at $\kappa = 0°$ (Figure 4a). At the 6-31G* level, the barrier to rotate from $\kappa = 0°$ to $\kappa = \pm 180°$ is about 10 kcal/mol. The energy difference between the cis and trans conformers is 9 kcal/mol, significantly higher than that for the nicotinamide and dihydronicotinamide carboxamide groups (2 kcal/mol and 4 kcal/mol, respectively). The computed energy minimum also shows good agreement with results from the X-ray crystallographic data base search (Figure 4b). Only seven structures containing the thiazole-4-carboxamide group were retrieved, all of them thiazole nucleosides.[33] These structures show that the carboxamide amino group tends to be cis-planar with the thiazole ring nitrogen. Observed κ values for the seven fragments lie in the range from −15° to +10°, with the exception of tiazofurin, for which $\kappa = 33.1°$. The larger value of κ observed in the tiazofurin crystal structure has been attributed to intermolecular packing interactions.[17]

The interactions stabilizing the cis ($\kappa = 0°$) conformation versus the trans ($\kappa = \pm 180°$) conformation in the thiazole-4-carboxamide group were studied by NBO analysis. The results are illustrated schematically in Figures 5e and 5f. Deletion of all charge transfer interactions between the thiazole ring and the carboxamide group yielded at −4.8 kcal/mol energy difference ($\Delta E_{CT} = E_{CT}$(cis) $- E_{CT}$(trans) = −4.8 kcal/mol). This is over half of the total energy difference between the cis and trans conformer ($\Delta E = E$(cis) $- E$(trans) = −9.3 kcal/mol). This charge transfer energy was further decomposed by deleting the interaction between the lone pair of electrons on the N3 atom and the antibonding orbital of the carboxamide amino N6–H6′ bond. Upon deletion, the energy of the cis conformer increased 2.6 kcal/mol more than that of the trans conformer. This indicates that the hydrogen bond-like interaction between the N3 lone pair and σ^*(N6–H6) orbital is the single largest source of charge transfer energy stabilizing the cis conformation. The non-charge transfer component of the energy difference is due to electrostatic and exclusion repulsion interactions. A large positive charge on H5 (0.29e) and a large negative charge on O6 (−0.66e) make these two atoms candidates for an electrostatic interaction which further stabilizes the cis conformation (Figure 5f). The trans conformation shows less charge transfer stabilization, with electrostatic repulsion between the negative carbonyl oxygen and thiazole nitrogen potentially destabilizing this conformation (Figure 5e).

Table I. Carboxamide Dihedral Angles κ(deg) from Crystal Structures of Enzyme-Bound Cofactors

PDB code	enzyme	resolution (Å)	cofactor[a]	ternary ligand	subunits	κ(deg)
6ADH	alcohol dehydrogenase (horse)	2.9	NAD^+	DMSO	2	31[b]
1HSD	3α,20β-hydroxysteroid dehydrogenase[c]	2.6	NAD(H)	solvent	4	34
1GD1	glyceraldehyde-3-phosphate dehydrogenase[d]	1.8	NAD(H)	sulfate	4	-22[b]
1GPD	glyceraldehyde-3-phosphate dehydrogenase (lobster)	2.9	NAD(H)	phosphate	2	0
3GPD	glyceraldehyde-3-phosphate dehydrogenase (human)	3.5	NAD(H)	phosphate	2	-27[b]
4MDH	cytoplasmic malate dehydrogenase (pig)	2.5	NAD(H)	sulfate	2	-16[b]
2LDB	lactate dehydrogenase[d]	2.8	NAD^+	*e*	1	11
1LDM	lactate dehydrogenase (dogfish)	2.1	NAD^+	oxamate	1	0
3LDH	lactate dehydrogenase (dogfish)	3.0	NAD^+	pyruvate	1	7
5LDH	lactate dehydrogenase (pig)	2.7	lac-NAD^+	citrate	1	-10
3DFR	dihydrofolate reductase (*L. casei*)	1.7	NADPH	methotrexate	1	4
8DFR	dihydrofolate reductase (chicken)	1.7	NADPH	calcium	1	0
8CAT	catalase (beef)	2.5	NADPH	*f*	2	-4
3GRS[g]	glutathione reductase (human)	2.0	NADPH	FAD	2	-169

[a] Oxidation state of cofactor is given only if stated explicitly by authors. [b] Average over noncrystallographic symmetry-related subunits. [c] *Streptomyces hydrogenans*. [d] *Bacillus steanothermophilus*. [e] D-fructose-1,6-biphosphate. [f] Protoporphyrin IX with Fe^{3+}. [g] Apo enzyme only. Coordinates for the complex not yet deposited in PDB. Carboxamide orientation is from ref 37.

Thus, both computational and crystallographic results suggest that the carboxamide group in the thiazole-4-carboxamide moiety is restricted to the trans conformation. This conformational restriction would be expected to have a significant effect on the dehydrogenase binding of the active anabolite TAD, as discussed next.

The Carboxamide Group in Enzyme–Cofactor Binding. Recall that the biological effects of the parent compound tiazofurin result from inhibition of the enzyme IMPd by the dinucleotide anabolite TAD.[1-5,10] The putative mechanism for this inhibition is that TAD mimics the binding of NAD(H) at the cofactor site.[15,16] Although the structure of IMPd is unknown, crystal structures of other dehydrogenase–cofactor complexes show that the carboximide group plays an important role in anchoring the reduced or oxidized nicotinamide ring to the enzyme.[22] We have seen that free nicotinamide and dihydronicotinamide carboxamide groups adopt either cis or trans conformations, while the thiazole-4-carboxamide group is restricted to the cis conformation. Given that this restriction would be expected to occur in TAD, it is clearly of interest to determine the carboxamide conformation required for dehydrogenase–cofactor binding. We have thus examined the crystal structures of all enzyme complexes found in the Protein Data Bank containing NAD^+, $NADP^+$, NADH, and NADPH. Results are summarized in Table I and Figure 6. For each structure, Table I lists the Protein Data Bank code, the corresponding enzyme, the resolution of the dataset, the bound cofactor and other ligands, the number of subunits and the conformation of the carboxamide group in the bound cofactor.[35] Figure 6 shows a histogram of the cofactor carboxamide angles κ listed in Table I.

It is apparent from Table I and Figure 6 that the carboxamide group of the enzyme-bound cofactor prefers the conformation in which its oxygen is cis to C4. Examination of the complexes indicates that, in most cases, specific hydrogen bonds between the cofactor site and the carboxamide group maintain this conformation (cf. Figure 8 in ref 22b). Recall that computational results showed this cis conformation ($\kappa = 0°$) to be about 2 kcal/mol less stable than the trans conformation ($\kappa = \pm 180°$). Further, crystal structures of free nicotinamide and dihydronicotinamide rings also indicated a preference for the trans conformer. Thus, the adoption of the higher energy cis conformation of the carboxamide group by enzyme-bound cofactor may be required for the biological function of the cofactor. In the dehydrogenases and reductases, this function is to transfer a hydrid ion between C4 and the substrate. Results from several investigators do in fact suggest that the hydride transfer reaction proceeds more readily for the cis conformation.[29,36] Thus the cis conformer may be favored for the bound cofactor due to the requirements of the enzyme's catalytic mechanism.[29,36] A possible exception to this observation is found in the structure of glutathione reductase, in which the carboxamide group of bound NADPH is in the trans conformation ($\kappa \sim -170°$).[37] In this enzyme, the nicotinamide moiety is stacked on the flavin adenine dinucleotide (FAD) prosthetic group, which acts as an intermediate in the hydride transfer.[37]

Discussion

Both computational and X-ray crystallographic data indicate that the reduced and oxidized nicotinamide moieties have two stable carboxamide group conformations in their unbound states; cis ($\kappa = 0°$) and trans ($\kappa = \pm 180°$). When enzyme-bound as the cofactors $NAD(P)^+$ and NAD(P)H, the cis conformation is preferred. The finding that the cis conformer is preferred by enzyme-bound cofactor is significant in studying dehydrogenase binding by TAD. As shown above, the thiazole-4-carboxamide group is constrained to the cis conformation. Thus, the carboxamide conformation in free TAD is likely constrained to that observed in enzyme-bound cofactor. This carboxamide group is also likely to play a role in dehydrogenase binding by TAD. The carboxamide group in the 4 position of the thiazole ring is required for activity,[38] and it has been suggested that TAD mimics cofactor binding to dehydrogenases.[15,16] However, the energy cost of rotating the carboxamide group of NAD(H) from the trans conformation favored in the free state to the cis conformer required in the enzyme-bound state can be significant (~2 kcal/mol). No such penalty exists for

(36) Cummins, P. L.; Gready, J. E. Mechanistic Aspects of Biological Redox Reactions Involving NADH 2: A Combined Semiempirical and Ab Initio Study of Hydride-ion Transfer between the NADH Analogue, 1-Methyl-Dihydronicotinamide, and Folate and Dihydrofolate Analogue Substrates of Dihydrofolate Reductase. *J. Comput. Chem.* **1990**, *11*, 791–804.

(37) (a) Pai, E. F.; Karplus, P. A.; Schulz, G. E. Crystallographic Analysis of the Binding of NADPH, NADPH Fragments, and NADPH Analogues to Glutathione Reductase. *Biochemistry* **1988**, *27*, 4465–4474. (b) Karplus, P. A.; Schulz, G. E. Substrate Binding and Catalysis by Glutathione Reductase as Derived from Refined Enzyme: Substrate Crystal Structures at 2Å Resolution. *J. Mol. Biol.* **1989**, *210*, 163–180.

(38) Jayaram, H. N.; Dion, R. L.; Glazer, R. I., Johns, D. G.; Robins, R. K.; Srivastava, P. C.; Conney, D. A. Initial Studies on the Mechanism of Action of a New Oncolytic Thiazole Nucleotide, 2-β-D-Ribofuranosylthiazole-4-Carboxamide. *Biochem. Pharmacol.* **1982**, *31*, 2371–2380.

TAD. This energy difference will contribute to the difference in enthalpy of binding between the two ligands. The requirements for cofactor binding to IMPd may be similar to those seen in the structures of other dehydrogenases. Thus, the fact that the carboxamide group in TAD is already locked into the bound conformation may explain in part its tighter binding to IMPd with respect to NAD^+.

It should be noted that TAD does bind to several other dehydrogenases with an affinity only comperable to that of NAD^+.[15] This indicates that carboxamide conformation is only one of a number of potential factors which will influence the enzyme binding of TAD. For example, weaker binding of TAD to several dehydrogenases has been attributed to a possible failure of these enzymes to maintain an energetically favored close sulfur–oxygen contact in the bound inhibitor.[15,18] If this interaction were not maintained by a particular binding site, it would be of sufficient magnitude to counter any advantage offered by the favorable carboxamide conformation in these enzymes. It is to be expected that dehydrogenase binding by a complex molecule will be influenced by multiple conformational factors. Nevertheless, constraint of carboxamide group rotation in the thiazole-4-carboxamide moiety will be one of these factors, and will have a significant effect on the binding of TAD to its target enzyme.

Acknowledgment. We thank Dr. Jeffrey P. Jones for helpful discussions and for introducing us to the NBO method. Work supported in part by Public Health Service Grant CA 45145.

Registry No. TAD, 83285-83-0; $NAD(P)^+$, 53-59-8; 4-thiazolecarboxamide, 3575-09-5; 1,4-dihydronicotinamide, 18940-08-4; nicotinamide, 98-92-0; dehydrogenase, 9035-82-9.

Supplementary Material Available: Citations for structures obtained from the Cambridge Structural Database (Table I) and from the Protein Data Bank (Table II) (7 pages). Ordering information is given on any current masthead page.

5,5-Disubstituted Hydantoins: Syntheses and Anti-HIV Activity

Robert N. Comber,* Robert C. Reynolds, Joyce D. Friedrich, Roupen A. Manguikian, Robert W. Buckheit, Jr., Jackie W. Truss, William M. Shannon, and John A. Secrist III

Organic Chemistry Department, Southern Research Institute, Birmingham, Alabama 35255-5305. *Received March 26, 1992*

A series of 5,5-disubstituted hydantoin derivatives was synthesized by alkylating 5,5-bis(mercaptomethyl)-2,4-imidazolidinedione (3) with various halomethylaromatic or halomethylheteroaromatic precursors, or by using the Buchener–Berg procedure on the required ketone. When evaluated for their ability to inhibit HIV-induced cell killing and virus production in CEM or MT-2 cells only compounds **2**, **4n**, **4o**, and **4i** demonstrated modest activity, the latter with an IC_{50} = 53 μM.

Introduction

Many strategies[1a-e] have been utilized in the design of new chemotherapeutic agents for the treatment of AIDS. Generally, new compounds have been designed to interfere with any of a number of key steps in the replicative cycle of the human immunodeficiency virus (HIV), the causative agent for this life-threatening disease. One strategy that has provided a number of promising compounds has been the disruption of virus adsorption to the host-cell membrane. This interaction is known to rely on an affinity of the virally-encoded glycoprotein gp120 for the cellular CD4 receptor of the host. Compounds that have been shown to interfere with this interaction include soluble forms of CD4, aurintricarboxylic acid, and various sulfated polysaccharides.[1b]

In 1986 Lehr and Zimmer reported that diphenylhydantoin (dilantin, 1), also a membrane-reactive drug that has been used in antiepileptic therapy for some 40 years, inhibits HIV binding to CD4 positive lymphocytes.[2] More recently, these findings have been extended to suggest that the aforementioned inhibition is likely due to host-cell membrane fluidization resulting in a reduced availability of the CD4 receptor for ligand interaction.[3] As a complement to this work, it has been demonstrated that dilantin suppresses the influx of Ca^{+2} ions that occurs shortly after HIV infection, suggesting a possible role of membrane-associated calcium-dependent cellular processes in HIV infection.[4] For several years we have had an interest in developing anti-AIDS drugs by targeting biological processes associated with the HIV glycoprotein coat. We have synthesized a number of polysaccharides[5] that were designed to interfere with the biosynthesis of gp120.

(1) This area has been reviewed by several authors including (a) De Clercq, E. Basic Approaches to Anti-Retroviral Treatment. *J. Acquired Immune Defic. Syndr.* **1991**, *4*, 207–218. (b) De Clercq, E. Targets and Strategies for the Antiviral Chemotherapy of AIDS. *Trends Pharmacol. Sci.* **1990**, *11*, 198–205. (c) Mitsuya, H.; Yarchoan, R.; Broder, S. Molecular Targets for AIDS Therapy. *Science* **1990**, *249*, 1533–1544. (d) Norbeck, D. W. Recent Advances in Anti-Retroviral Chemotherapy for AIDS. *Annu. Rep. Med. Chem.* **1990**, *25*, 149–158. (e) Hirsch, M. S. Chemotherapy of Human Immunodeficiency Virus Infections: Current Practice and Future Prospects. *J. Infect. Dis.* **1990**, *161*, 845–857.

(2) Lehr, H. A.; Zimmer, J. P. Diphenylhydantoin zur Prophylaxe und Therapie von AIDS. *DMW, Dtsch. Med. Wochenschr.* **1986**, *111*, 1001–1002.

(3) Lehr, H. A.; Zimmer, J. P.; Hübner, C.; Ballmann, M.; Hachmann, W.; Vogel, W.; Baisch, H.; Hartter, P.; Albani, M.; Kohlschütter, A.; Schmitz, H. Decreased Binding of HIV-1 and Vasoactive Intestinal Peptide Following Plasma Membrane Fluidization of $CD4^+$ Cells by Phenytoin. *Virology* **1990**, *179*, 609–617.

(4) Cloyd, M. W.; Lynn, W. S.; Ramsey, K.; Baron, S. Inhibition of Human Immunodeficiency Virus (HIV-1) Infection by Diphenylhydantoin (Dilantin) Implicates Role of Cellular Calcium in Virus Life Cycle. *Virology* **1989**, *173*, 581–590.

(5) Comber, R. N.; Secrist, J. A., III; Schutzbach, J. S.; Manguikian, R. A. Thio-Linked Mannopolysaccharides: Synthesis and Anti-HIV Activity. Manuscript in preparation. (b) Comber, R. N.; Secrist, J. A., III; Dunshee, D. A.; Friedrich, J.; Petty, S. Thio-Linked Glucopolysaccharides: Synthesis and Anti-HIV Activity. Manuscript in preparation.

0022-2623/92/1835-3567$03.00/0