Naturally Occurring 5-Deazaflavin Coenzymes: Biological Redox Roles

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Riboflavin (Vitamin B_2) with its tricyclic isoalloxazine ring is perhaps the most versatile biological redox reagent. In its 5′-phosphate (FMN) and 5′-ADP (FAD) coenzymatic forms, it sits at the crossroads of many enzymic redox routes as a $1e^-/2e^-$ redox switch.[1,2] The ability of riboflavin to interface between obligate two-electron-transfer agents (e.g., NADH) and one-electron-transfer agents, (e.g. iron or molecular oxygen) results from the kinetic and thermodynamic accessibility of the one electron-reduced flavin semiquinone under biological conditions. To dissect out the contributions of the N5-nitrogen (in the pyrazine ring) and the N1-nitrogen (in the uracil ring)[3,4] in controlling the accessibility of the semiquinone form of flavin coenzymes, we have, in collaboration with colleagues at Merck,[5] previously analyzed the redox competence of synthetic riboflavin analogues such as 5-carba-5deaza-riboflavin ($2e^-$ steps only), 1-carba-1-deazaflavin $1e^-$ and $2e^-$ steps), and 1,5-dideazariboflavin (redox incompetent) shown in Table I. The 5-substituent is the crucial determinant for one-electron chemistry since the carbon for nitrogen substitution restricts the 5-deaza system to hydride transfer only. By contrast, the 1-carba analogue converts the uracil ring to deazauracil and makes the tricycle more difficult to reduce but still able to facilitate both $2e^-$ and $1e^-$ redox reactions.[1,4]

Although 5-deazariboflavin was first synthesized in 1970,[6] this flavin analogue attracted much more of our attention with the report of Eirich in 1978[7] that a 5-deazariboflavin was a naturally occurring structure and in fact was a predominant cellular constituent in anaerobic bacteria that biosynthesize methane. These fastidiously anaerobic methanogenic bacteria are ancient and are thought to be descendants of primordial organisms. They have been designated members of the Archaebacterial kingdom, a newly defined "third form" of life[8] (vs. eukaryotes and eubacteria). Eirich, Vogels, and Wolfe termed the molecule coenzyme F_{420} (Chart I) because of its intense aborbance at 420 nm ($\epsilon 420$ = 40,000 M^{-1} cm^{-1}). Coenzyme F_{420} is an 8-hydroxy-7-desmethyl-5-deazariboflavin derivative where the 5′-ribityl-OH group is phosphorylated, as in FMN, but is instead in phosphodiester linkage with a lactyl oligoglutamyl grouping (reminiscent of the folate coenzyme oligoglutamyl side chains). The number of glutamates varies among various methanogenic bacteria and also in *Steptomycetes* (vide infra). Coenzyme F_{420} is a signature molecule for methanogenic bacteria and is present at up to 100 mg/kg of cells.

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Table I
(Carbon) Deaza Analogues of Riboflavin

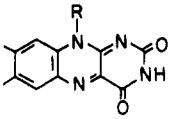
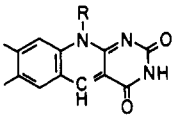
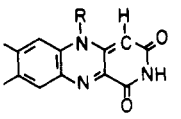
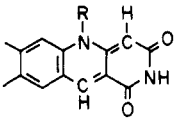
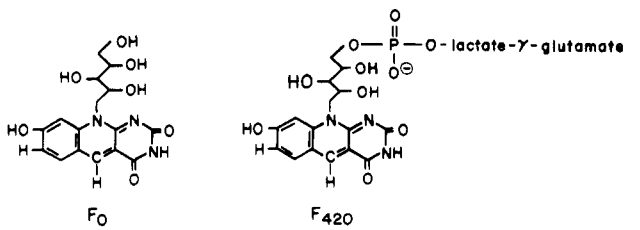
structure	species	$E^{\circ\prime}$	coenzymatic competence
	riboflavin	−210 mV	$1e^-$ and $2e^-$
	5-deazariboflavin	−310 mV	$2e^-$ only
	1-deazariboflavin	−280 mV	$1e^-$ and $2e^-$
	1,5-dideazariboflavin	−370 mV	incompetent

Chart I



(up to 100mg/kg of methanogenic bacteria)

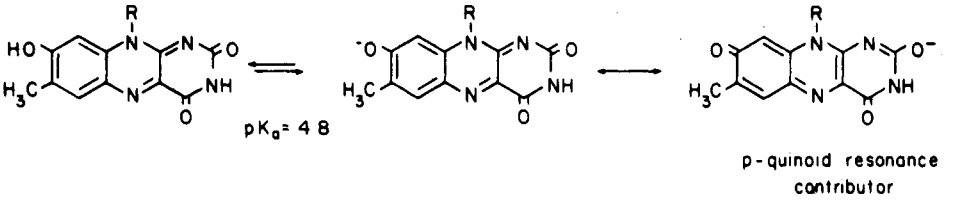
The 8-hydroxy-7-desmethyl-5-deazaribovlavin[8] itself, termed F_0, is excreted into culture medium by some methanogens. The 420-nm chromophore is associated with the 8-hydroxy group and is precedented by chromophoric changes with 8-hydroxyriboflavin where phenol ionization (pK_a = 4.8) generates a highly colored delocalized anion (λ_{max} = 490 nm) (Scheme I). The corresponding phenolic pK_a in synthetic 8-hydroxy-5-deazariboflavin and in F_0 (8-hydroxy-7-desmethyl-5-deaza) is 5.8[7,10] so F_{420} is 99% the delocalized chromophoric anion at pH 7.8. On the acid side the 8-

(1) Walsh, C. *Acc. Chem. Res.* **1980**, *13*, 148.
(2) Massey, V.; Hemmerich, P. *Biochem. Soc. Trans.* **1980**, *8*, 246.
(3) Walsh, C.; Fisher, J.; Spencer, R.; Graham, D.; Ashton, W.; Brown, J.; Brown, R.; Rogers, E. *Biochemistry* **1978**, *17*, 1942.
(4) Walsh, C.; Jacobson, E.; Ryerson, C. *Adv. Chem. Ser.* **1980**, *34*, 179.
(5) Ashton, W.; Graham, D.; Brown, R.; Rogers, E. *Tetrahedron Lett.* **1977**, 2551.
(6) O'Brien, D.; Weinstock, L.; Cheng, C. *J. Heterocycl. Chem.* **1970**, *7*, 99.
(7) Eirich, D.; Vogels, G.; Wolfe, R. *Biochemistry* **1978**, *17*, 4583.
(8) Woese, C.; Magrum, L.; Fox, G. *J. Mol. Evol.* **1978**, *11*, 245.
(9) Ashton, W.; Brown, R.; Jacobson, F.; Walsh, C. *J. Am. Chem. Soc.* **1979**, *101*, 4419.
(10) Jacobson, F.; Walsh, C. *Biochemistry* **1984**, *23*, 979.

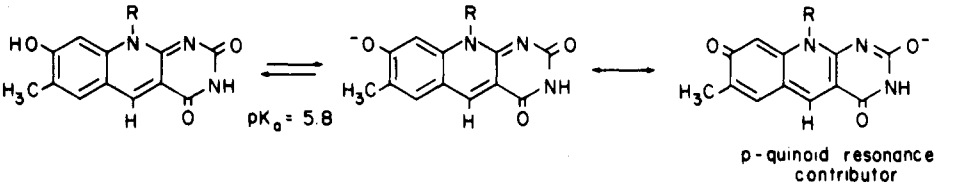
0001-4842/86/0119-0216$01.50/0

Scheme I

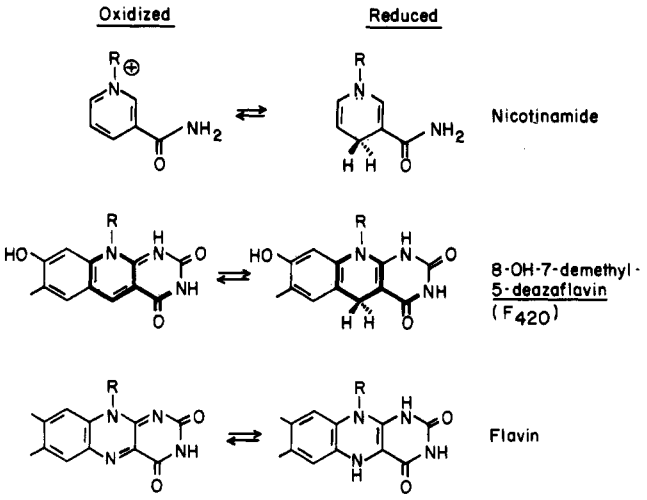
8-Hydroxyriboflavin



8-Hydroxy-5-deazariboflavin



Scheme II



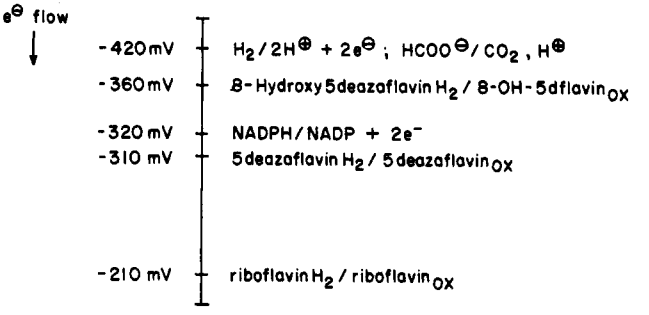
hydroxy-5-deazaflavin system has a λ_{max} of 390 nm with $E = 10^4\ M^{-1}\ cm^{-1}$. The electron-rich anionic F_{420} species is difficult to reduce and F_{420}-redox enzymes may protonate the *p*-quinonoidal anion at their active sites to facilitate reduction.

Although F_{420} and F_0 are tricyclic analogues of riboflavin, the 5-carba substitution converts the central pyrazine ring to a pyridine and the structural analogy to NAD-type nicotinamide coenzymes is dramatic as outlined in Scheme II. This pyrazine to pyridine transform (in oxidized state) and corresponding dihydropyrazine to dihydropyridine change in the two-electron-reduced state dominates redox function. The pyridine radicals are much higher in energy than the pyrazine radicals (of flavins), ensuring that both the 8-OH-5-deazaflavins and the nicotinamides are obligate two-electron, hydride-transfer coenzymes in ground-state chemistry.[10,11] It is therefore fruitful to think of F_{420} as a nicotinamide in flavin's clothing. Thus both F_{420} and NAD are low-potential, prochiral, air-stable hydride donors in the reduced state (Chart II). Compared to riboflavin, the 5-carba and 8-OH substitutions have ratcheted the redox potential 150 mV more negative, −210 mV to −360 mV,[7,10] (Scheme III), and so F_{420} sits at an entirely different redox crossroads from flavin coenzymes. Whereas flavins are reduced by NADH, $F_{420}H_2$ instead reduces NAD or NADP. In fact with a redox potential of −360mV, the $F_{420}/F_{420}H_2$ couple is poised halfway between the $H_2/2H^+$ (the hydrogen electrode) and $HCOO^-/CO_2$ couples (−420 mV each) and the NADP/NADPH couple (−320 mV) and this presages the function of three F_{420}-utilizing methanogen enzymes as discussed below.

Chart II
8-OH-5 Deazaflavins vs. NADH

Hydride transfer	Hydride transfer
$E^{o\prime} = -360$mV	$E^{o\prime} = -320$mV
dihydro form relatively stable to autoxidation	dihydro form stable to autoxidation
dihydro form: C_5 (of nicotinamide) is prochiral	dihydro form: C_4 is prochiral

Scheme III



Redox Roles for Coenzyme F_{420} in Methanogens

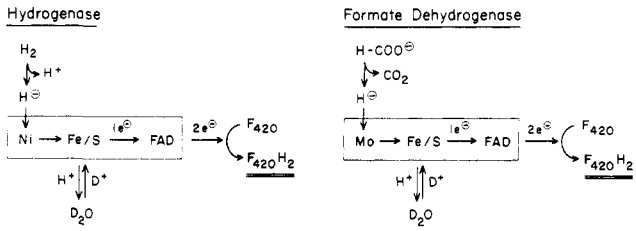
To set briefly the biological context for function of the low-potential hydride-transfer coenzyme F_{420} in methanogenic bacteria, we note that methanogens sit at the end of the food chains in the anaerobic digestion of biomass. Methanogenic bacteria can live chemoli-

(11) Yamazaki, S.; Tsai, L.; Stadtman, T.; Jacobson, F.; Walsh, C. *J. Biol. Chem.* **1980**, *255*, 9025.

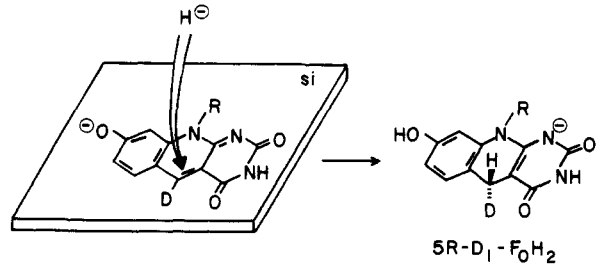
Table II
Coenzyme F_{420} and Proposed Redox Roles in Metabolism of Methanogenic Bacteria

1. $H_2 + F_{420} \rightleftharpoons F_{420}H_2$	hydrogenase
2. $NADPH + F_{420} \rightleftharpoons NADP + F_{420}N_2$	nicotinamide–deazaflavin transhydrogenase
3. $HCOO^{\ominus} + F_{420} + H^{\oplus} \rightleftharpoons CO_2 + F_{420}H_2$	formate dehydrogenase
4. $CO_2 + \text{CH}_3\text{COSCoA} + F_{420}H_2 \rightleftharpoons \text{CH}_3\text{COO}^{\ominus} + {}^{\ominus}\text{SCoA} + F_{420}$	pyruvate synthase
5. (?) $CH_3SCoM + F_{420}H_2 \rightleftharpoons \boxed{CH_4} + F_{420}$ (ATP, several proteins)	methyl CoM reductase

Scheme IV



Scheme V



thoautotrophically on CO_2 and H_2 or they can dismutate acetate to CO_2 and CH_4. For calibration, about 20% of the flux to make methane is from CO_2 and about 70% from acetate in a municipal biomass waste digester. The estimated annual global amount of methane generated microbially is about 10^{15} g, (10^9 tons), so methane biogenesis is large-scale biochemistry.[12] For the eight-electron reduction of CO_2 to CH_4, 4 H_2 molecules are oxidized by action of hydrogenase enzymes. The methanogenic bacteria contain at least six novel coenzymes, several involved in the $CO_2 \rightarrow CH_4$ pathway (e.g. methanofuran,[13] methanopterin,[14] methyl CoM,[15] the nickel-containing tetrapyrrolic coenzyme F_{430}[16,17,18] and three novel nickel-containing enzymes). The nickel is tightly and stoichiometrically bound in each enzyme. One such enzyme is the F_{420}-reducing hydrogenase,[19] the second a methyl-*S*-coenzyme M reductase,[20] the third a carbon monoxide dehydrogenase involved in acetate synthesis.[21] Intense efforts are underway to characterize ligand structure and nickel redox function in these three enzymes which seem to be a nickel hydrogenation catalyst, nickel desulfurization catalyst, and nickel biocarbonylation catalyst, respectively.

Five proposed roles for F_{420} in methanogen metabolism are summarized in Table II. The F_{420}-reducing hydrogenase,[19,23] the F_{420}-reducing formate dehydrogenase,[24] and the NADP-F_{420} oxidoreductase[25] have been purified to homogeneity while purported roles for $F_{420}H_2$ in driving reductive carboxylation of acetyl CoA[26] and of methyl thioether cleavage to methane[20] have only been demonstrated in crude cell-free extracts.

The simplest enzyme, the transhydrogenase, catalyzes a direct hydrogen transfer from one of the C_5 prochiral methylene hydrogens of the dihydro form of F_{420} directly to C_4 of NADP in an apparent hydride transfer.[11] By contrast D_2 and $DCOO^-$ do not yield 5-deuterio dihydro F_{420} in hydrogenase[27] or FDH-mediated reactions (see Scheme IV).[28] The itinerant hydride (deuteride) equivalent exchanges with solvent protons at some stage in catalysis as demonstrated by production of chiral 5-D_1-F_0H_2 species in D_2O. The absolute chirality of these enzymically generated monodeutero dihydrodeazaflavins has recently been established by Yamazaki et al[29,30] by degradation to hydroxyethylquinolineone and ORD comparison to authentic deuterated samples. For all three F_{420}-utilizing enzymes the incoming hydride equivalent is added to the Si face at the C_5 locus of the 8-hydroxy-5-deazaflavin substrate as depicted in Scheme V.

The F_{420}-reducing hydrogenase and formate dehydrogenase each have three subunits and three associated redox centers; the H_2ase[23] has nickel, iron–sulfur clusters, and FAD while the FDH[31] has molybdenum, iron–sulfur clusters, and FAD (Scheme IV). It is likely that the nickel site and the molybdenum site respectively are initial sites for *hydride transfer in* from H_2

(12) Whitman, W. In *The Arachaebacteria*; Wolfe, R.; Woese, C., Eds.; 1984, Academic: New York, 1984.
(13) Leigh, J.; Rinehart, K.; Wolfe, R. *J. Am. Chem. Soc.* **1984**, *106*, 3636.
(14) Keltjens, J.; Daniels, L.; Johnson, H.; Borm, P.; Vogels, G. *Eur. J. Biochem.* **1983**, *130*, 545.
(15) Taylor, C.; Wolfe, R. *J. Biol. Chem.* **1974**, *249*, 4877.
(16) Gunsalus, R.; Wolfe, R. *FEMS Microbiol. Lett.* **1970**, *3*, 191.
(17) Diekert, G.; Jaenchen, R.; Thauer, R. *FEBS Lett.* **1980**, *119*, 118.
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(20) Ellefson, W.; Wolfe, R. *J. Biol. Chem.* **1980**, *255*, 8388.
(21) Krzycki, J.; Zeikus, J. *J. Bacteriol.* **1984**, *188*, 231.
(22) Nelson, M.; Ferry, J. *J. Bacteriol.* **1984**, *160*, 526.
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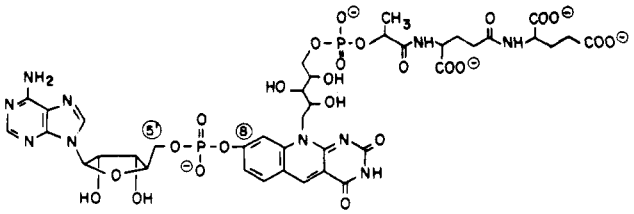
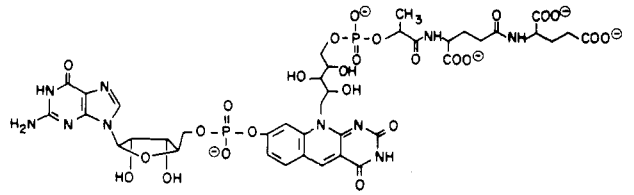
(24) Schauer, N.; Ferry, J. *J. Bacteriol.* **1983**, *155*, 467.
(25) Yamazaki, S.; Tsai, L. *J. Biol. Chem.* **1980**, *255*, 6462.
(26) Zeikus, J.; Fuchs, G.; Kenealy, W.; Thauer, R. *J. Bacteriol.* **1977**, *132*, 604.
(27) Fox, J.; Orme-Johnson, W. H.; Walsh, C.; Livingston, D. *Biochemistry*, submitted.
(28) Honek, J.; Schauer, N.; Ferry, J.; Orme-Johnson, W. H.; Walsh, C. *Biochemistry*, submitted.
(29) Yamazaki, S.; Tsai, L.; Stadtman, T.; Teshima, T.; Nakaji, A.; Shiba, T. *P.N.A.S.* **1985**, *82*, 1364.
(30) Teshima, T.; Nakaji, A.; Shiba, T.; Tsai, L.; Yamazaki, S. *Tetrahedron Lett.* **1985**, 351.
(31) Schauer, N.; Ferry, J. *J. Bacteriol.* **1982**, *150*, 1.

Chart III
Structure of Methanogen F_{390}'s



F_{390}-A



F_{390}-G

or from $HCOO^-$. The Fe/S centers are likely one-electron conduits to the bound FAD from which, at the $FADH_2$ level, *hydride transfer out* occurs to C_5 of F_{420}. The bound FAD in each enzyme is the required $1e^-/2e^-$ redox switch, interfacing between the one electron Fe/S clusters and the two electron-accepting 8-hydroxy-5-deazaflavin substrate. The net hydride-transfer process is obscured in both hydrogenase and formate dehydrogenase catalysis by proton exchange with solvent which could occur at any of the three redox sites in either enzyme, for example from N5 of bound FAD_2 as one case. Indeed, it is known that intact methanogenic bacteria growing on CO_2 and D_2 in H_2O generate CH_4 almost quantitatively,[32] consistent with the above data on the hydrogenase. Hydrogenase from which FAD has been removed can still reduce one-electron acceptors (methyl viologen) but not F_{420} until FAD has been added back.[24] The FAD bound in hydrogenase and formate dehydrogenase must have oxidized FAD bound more tightly than $FADH_2$, to lower bound FAD redox potential from −210 mV towards the $F_{420}/F_{420}H_2$ couple (−360 mV). This is precented in flavoproteins such as flavodoxins, where the one-electron flavin potential is ca. −400 mV.

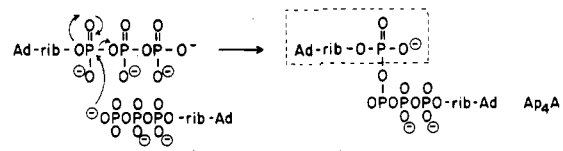
In oxidizing H_2 or $HCOO^-$ and passing on the two electron equivalents to generate $F_{420}H_2$, methanogenic bacteria generate a kinetically stable, diffusable, low potential energy currency to spend in driving cellular metabolism. By passing electrons to F_{420} (−360 mV) rather than to NADP (−320 mV) directly, the bacteria harvest an additional 1.6 kcal/mol in the energy available in H_2 or $HCOO^-$ oxidation and so make a better living with this finely tuned 8-hydroxy-5-deazaflavin energy currency.

F_{420} in an Oxidant Stress Response?

When the fastidiously anaerobic methanogenic bacteria are exposed to O_2, they are rapidly inactivated. On exposure of intact cells (but not cell-free extracts) to air, the F_{420} content (as high as 100 mg/kg) of the cells rapidly declines.[33] While this was thought initially to

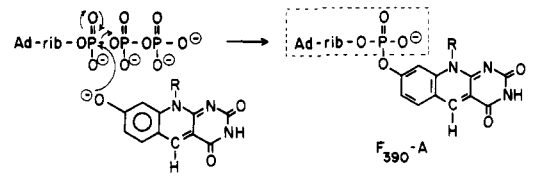
Scheme VI

1. Adenylyl Transfer to ATP in Synthesis of Oxidant Stress Alarmone AppppA
(Bruce Ames et al)

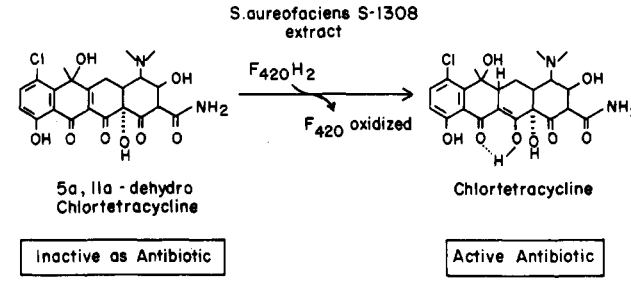


Catalyzed by various bacterial aminoacyl t-RNA synthetases

2. Proposed Adenylyl Transfer in Conversion of $F_{420} \rightarrow F_{390}$-A Under Oxidant Stress



Scheme VII
The Last Step in Chlortetracycline Biosynthesis in Streptomyces Aureofaciens



$F_{420}H_2$ is the specific reductant (initially termed "cosynthase I")

be some F_{420} destructive event, we have shown that as F_{420} declines two reciprocally accumulating F_{390} chromophores are the 8-0-AMP and 8-0-GMP esters of F_{420}[34] (Chart III). These adenylylated and guanylylated F_{420} molecules are not hydrogenase substrates and may signal the cells to shut down energy metabolism in response to oxidative stress. An analogy in these Archaebacteria may be derived to recent work by Ames and colleagues in eubacteria subjected to oxidant stress.[35] Salmonella rapidly synthesize Ap4A, possibly as an "alarmone" regulatory signal to selectively shut down certain metabolic circuits (Scheme VI, eq 1). Ap 4A is generated by adenylyl (AMP fragment) transfer from one ATP to a second ATP in the active site of an aminoacyl transferase. The adenylylation of the phenoxide nucleophile of F_{420} could be a corresponding Archaebacterial alarmone response in F_{390A} generation (Scheme VI, eq. 2). F_{390A} may also be a primordial variant of an AMP–O-tyrosyl regulatory strategy seen in eubacterial and eukaryotic metabolism.

F_{420} Redox Roles in Non-Methanogenic Organisms

While one could perhaps initially regard 8-hydroxy-5-deazaflavins as an evolutionary cul de sac in coenzyme evolutionary development adapted for the low potential metabolism of methanogen life, it turns out that F_{420} has also been detected in *Streptomycetes*,[36] in mycobacteria,[37] in halobacteria,[38] and in the blue-gree alga

(32) Spencer, R.; Daniels, L.; Orme-Johnson, W. H. *J. Bacteriol.* **1980**, *141*, 694.
(33) Schonheit, P.; Keweloh, H.; Thauer, R. *FEMS Microbiol. Lett.* **1981**, *12*, 347.
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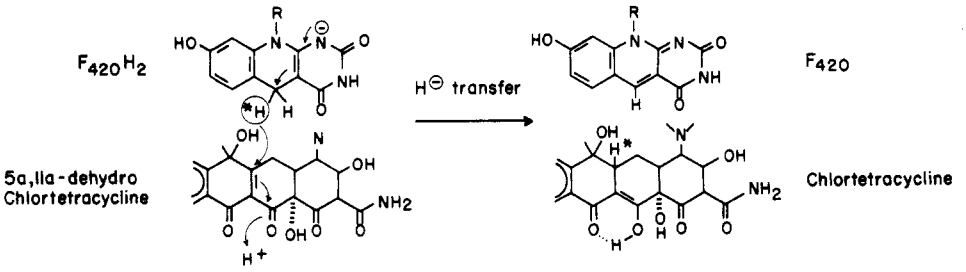
(35) Lee, P.; Bochner, B.; Ames *J. Biol. Chem.* **1983**, *258*, 6827.
(36) Eker, A. *Photochem. Photobiol.* **1980**, *32*, 593.

Scheme VIII
Possible Mechanism for Chlortetracycline Formation: $F_{420}H_2$ as a Hydride Donor for Conjugate Addition



Anacystis nidulans[36] in the 7 years since its initial identification.

Two remarkable and distinct coenzymatic roles have been documented to date in *Streptomycetes* metabolism and are summarized below.

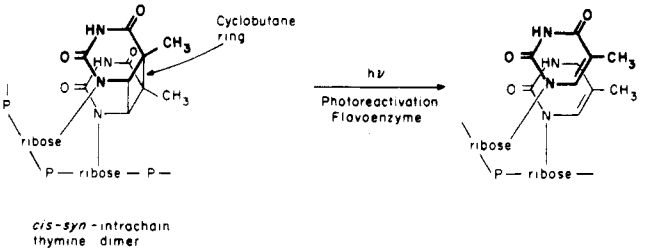
F_{420} as Redox Coenzyme in the Last Step of Tetracycline Biosynthesis

In 1960 McCormick and colleagues at Lederle,[39] working on the biosynthesis of the antibiotic chlortetracycline in blocked mutant strains of *Streptomycetes aureofaciens*, showed that conversion of the penultimate metabolite 5a,11a-dehydrochlortetracycline (inactive as an antibacterial) to the active chlortetracycline (Scheme VII) required a small diffusable molecule they termed "cosynthase I". They reported a UV–visible spectrum (420 nm chromophore) of cosynthase I and an elemental analysis (that was not quite correct) and did not identify the then unknown structure. Subsequent to the initial studies reported on F_{420} and F_0[7,9], we gave the Lederle group a synthetic sample of F_0, and they confirmed that cosynthase I was a triglutamyl F_{420} species.[40] While specific studies with 5-[^{3}H]-$F_{420}H_2$ remain to be done it is quite likely that dihydro F_{420} is acting as a specific hydride-transfer donor in 1,4-addition to the enone system in enzymic conversion of inactive precursor to active chlortetracycline as shown in Scheme VIII. There is indirect evidence that $F_{420}H_2$ fulfills the same role in the last step of oxytetracycline biosynthesis[41] in other strains of *Streptomycetes* and these may be the organisms of choice for analysis of the pathway of F_{420} biosynthesis since those genes may be clustered in these bacteria and amenable to cloning and overproduction.

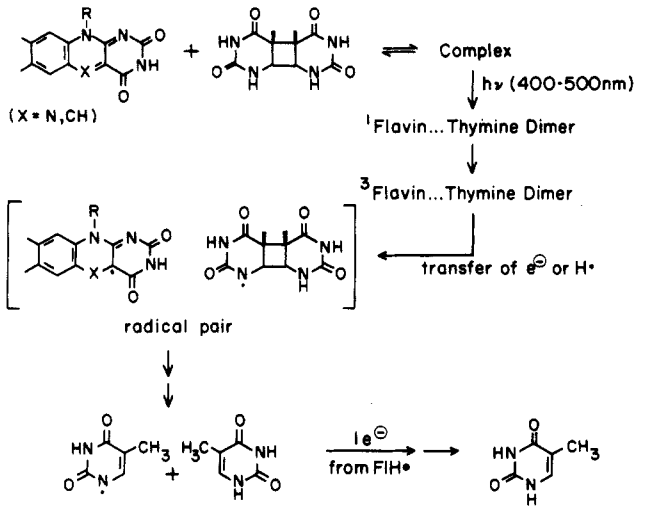
F_{420} as Coenzyme in Enzymic Photoreversion of *cis-syn*-Cyclobutane Dimers in UV-Damaged DNA

The most prevalent covalent change in DNA after absorption of UV light is the formation of cyclobutane rings as two adjacent intrachain thymine residues photodimerize to *cis-syn*-thymine dimers (T<>T) (Scheme IX). Enzymic repair is crucial for organism survival and maintenance of DNA information content, and repair occurs both in dark and light-mediated processes. The dark reaction excision–repair enzyme

Scheme IX



Scheme X
Possible Mechanism for Flavins, 5-Deazaflavins as Sensitizers for Dimer Photomonerization



systems are common and efficient for many types of DNA adducts,[42] including T <> T repair, while photoreversion is essentially limited to T <> T repair. Photoreversion enzymes use visible light, at wavelengths of 360–440 nm where nucleic acids do not absorb, to drive the net retro 2 + 2 cycloreversion of the cyclobutane-containing T <> T dimers in an apparent photosensitized process. Two types of photoreactivation enzymes have been purified recently. Enzymes from *E. coli*[43,44] and yeast[45] contain FMN while that from *Streptomycetes griseus*[46] has stoichiometrically bound coenzyme F_{420} and has a photoactivation spectrum at 420 nm as does the photoreaction enzyme from the blue green alga *Anacystis nidulans*. Eker noted 7% nonenzymic photomonomerization of T<>T by F_0, and we have recently shown several flavins and 5-deazaflavin can act, in the

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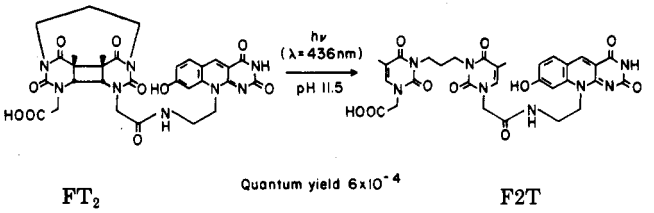
Scheme XI
Intramolecular Photosynthesized Cleavage by 8-Hydroxy-5-Deazaflavin of a Covalently Tethered *cis-syn*-Thymine Dimer Is Successful



FT_2 F2T

absence of enzyme, as catalytic photosensitizers of the cycloreversion process.[47]

In contrast to ground-state chemistry, in the excited state both the flavin *and* 5-deazaflavins have accessible semiquinone states and 5-deazariboflavin is a broadly useful, low-potential, one-electron photoreductant (e.g., for difficulty reducible redox proteins).[48] A possible route for flavin or 5-deazaflavin photosensitized reversion of T <> T is indicated in Scheme X, postulating one-electron transfer from T <> T dimer to yield the thymine radical cation and (deaza)flavin semiquinone complex. Radical fragmentation and back electron transfer would complete photomonomerization.[47] Although our model system was successful with 5-deazariboflavin and 8-methoxy-5-deazariboflavin, we had no success in irradiation with the natural 8-hydroxy-5-deazaflavin chromophore in intermolecular photosensitizations—possibly due to the rapid, competing photodestruction of F_0. We have recently prepared an intramolecularly tethered *cis-syn*-thymine dimer-8-hydroxy-5-deazaflavin species[49] shown in Scheme XI. On irradiation of the dimeric FT_2 at the deazaflavin λ_{max} (426 nm), T <> T intramolecular monomerization, to the cleaved, rearomatized F2T, is achieved albeit at low quantum yield (6×10^{-4}). These experiments begin to map out the role of the excited state of the 8-OH-5-deazaflavin coenzymes in the biologically crucial and chemically interesting retro 2 + 2 repair of thymine dimers in DNA.

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Concluding Remarks

The vitamin B_2-based flavin coenzymes have been known and studied for over 50 years and have been found to be among the most versatile biological redox catalysts, for electron transfers, for substrate dehydrogenations and for reductive activations of dioxygen. The natural 5-deazaflavins have been identified only since 1978 but have already been detected in some unusual biological redox niches. The tricyclic 5-deazaisoalloxazine nucleus is a functional hybrid (and perhaps an evolutionary one) between monocyclic nicotinamide and tricyclic flavin coenzymes. In the ground state the 8-hydroxy-5-deazaflavin coenzymes appear to be low potential variants of the nicotinamide-type hydride transfer coenzyme. They are perfectly suited to harvest energy from gaseous H_2 in hydrogenase catalysis. As a nicotinamide mimic F_{420} allows detection of direct hydrogen transfer and stereochemical outcome at the prochiral C_5 methylene locus in the dihydro oxidation state. On photo excitation, the λ_{max} is in the blue-green region of the visible spectrum, the one-electron-reduced deazaflavin semiquinone becomes kinetically accessible and is a stronger one-electron-reducing agent than the corresponding flavin semiquinone. This property is well-tailored to function as photosensitizing coenzyme to initiate a radical pathway for cleavage of the cyclobutane-containing thymine dimers in UV-damaged DNA by the photoreversion enzyme, DNA photolyase. The 5-deazaflavin structure may have been an early experiment in redox coenzyme evolution to yield a molecule with properties hydrid between nicotinamide and flavin redox coenzymes.

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Registry No. Coenzyme F_{420}, 64885-97-8.