bridging and P–O fission are occurring in the transition state or combination of transition states that determine the rate. It is very unlikely that proton bridging to the leaving group oxygen is occurring because 4-nitrophenol is substantially dissociated at this pH.[10] Thus proton bridging is probably occurring between the enzymic histidine and the serine nucleophilic oxygen, implying that O–P bond *formation* to the nucleophile is also rate-limiting in addition to fission of the P–O bond to the leaving group. We cannot say whether these processes are concerted[11] or stepwise.

These and other data are beginning to delineate the structural features that the enzyme–phosphonylation transition state must possess. In the near future, these structural features will be introduced by molecular-modeling techniques[12] into the known active-site structures of serine hydrolases, to deduce the molecular origins of the phenomenon of catalytic recruitment.

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Biosynthesis of 3,6-Dideoxy Hexoses: C-3 Deoxygenation May Proceed via a Radical Mechanism

Oksoo Han and Hung-wen Liu*

Department of Chemistry, University of Minnesota Minneapolis, Minnesota 55455

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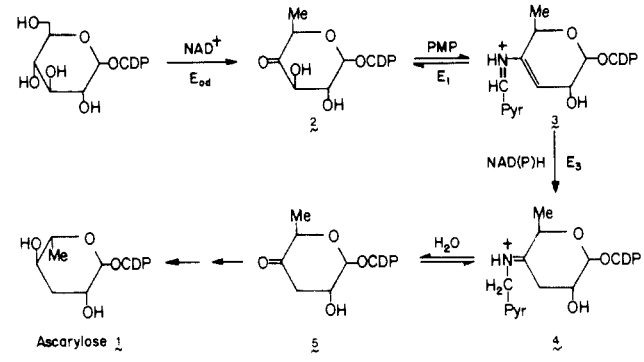
The 3,6-dideoxyhexoses are found in the lipopolysaccharide of gram-negative bacteria where they have been shown to be the dominant antigenic determinants.[1] On the basis of the pioneering efforts of Strominger and his co-workers,[2] the pathway for the biosynthesis of ascarylose, a 3,6-dideoxy-L-*arabino*-hexose **1**, had been put forward as shown in Scheme I. The key reaction of this proposed sequence is the C-3 deoxygenation step catalyzed by enzymes E_1, a pyridoxamine-5′-phosphate linked enzyme, and E_3, a NADPH dependent catalyst, both of which had been purified from *Pasturella pseudotuberculosis*.[3] Although the catalytical roles of these enzymes have been well defined,[2] the intimate mechanism of these steps is still disputable. For instance: (1) the hypothesized 3,4-glucoseen product **3** has never been identified; therefore, the actual mechanism of E_1 remains unresolved;[2c,4] (2) the E_3-catalyzed reduction of **3** which is still E_1 bound is believed to be a hydride-transfer process; however, such a reduction demands that their active sites be brought into closer proximity than is possible;[5] (3) incubation of E_3 with [4-$^3H_2$]NADPH resulted

Scheme I



in no tritium incorporation at either the product **5** or the regenerated PMP coenzyme; moreover, both the 4R and 4S hydrogens of NADPH were found to be labile in this reduction step;[2d] (4) E_3 can also catalyze the direct electron transfer from NADH to O_2;[2d,3b] albeit it contains no chromophoric groups. In an effort to clarify these mechanistic ambiguities, we have isolated an "E_3 equivalent" from *Yersinia pseudotuberculosis*[7] which is known to have ascarylose as the nonreducing terminal sugar in its lipopolysaccharide structure.[8] Reported herein is the preliminary characterization of the catalytic properties of this enzyme as a NADH oxidase and the consequent implication on its mechanism as a 3,4-glucoseen reductase.[9]

The purified enzyme[10] consists of a single polypeptide chain with a molecular weight of 41 000 and contains no metals.[11] Its UV–vis spectrum is that of a simple polypeptide with an absorption maximum around 280 nm. This result unequivocally demonstrates that this enzyme is not a flavoprotein and possesses none of the common electron carriers to mediate the electron transfer from NADH to O_2. The nature of the oxygen metabolite was determined to be H_2O_2 based on the ferrous-ferric procedure of Thurman et al.[12] and the oxidation of *leuco*-2′,7′-dichlorofluorescein (*leuco*-DCF) to DCF of Kochi and Wartburg.[13] Since the ratio of NADH oxidized to H_2O_2 produced is approximately one, this enzyme-catalyzed NADH oxidation is clearly a two-electron redox process overall. A variety of alternate electron

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(10) A highly selective sequence incorporating DEAE cellulose, phenyl-Sepharose, DEAE Sephadex, and Sephadex G-100 chromatography was developed in this study which led to a 2000-fold purification of this protein. Overall yield was ca. 4 mg of enzyme per 480 g of wet cells.
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acceptors were examined to test their competence as oxidants for this enzymatic reaction. It was found that dichlorophenolindolphenol (DCPIP) was the most potent electron acceptor tested,[14] while flavin analogues show no effect on catalysis. The latter finding agrees well with the earlier assessment that the NADH oxidase activity is flavin independent. However, the revelation of ferricytochrome *c* and ferricyanide as effective mediators was most intriguing since both compounds are well-known one-electron oxidants. The stoichiometry of NADH oxidation versus ferricyanide reduction was consequently shown to be 1.98. These observations strongly suggest that the enzyme-catalyzed H_2O_2 formation is not a direct two-electron reduction of molecular oxygen but may instead be a one-electron reduction process followed by dismutation of the nascent superoxide ($2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$).

The NADH dependent $O_2^{\bullet-}$ generating activity was assessed by measuring the superoxide dismutase inhibitable reduction of ferricytochrome *c*.[15] The experimental results clearly show that one out of the five Fe(III) being reduced in this assay received its electron from $O_2^{\bullet-}$.[16] Although such single-electron transfer to and from O_2 accounts for only 20% of the total reduction flux, this may simply reflect its minimum contribution in facilitating electron egress in the redox process. Since the active enzyme is metal-free, the indisputable formation of $O_2^{\bullet-}$ as the proximate reducing intermediate suggests the participation of an enzyme-bound organic cofactor mediating the obligatory $2e^-/1e^-$ conversion as electrons pass on from NADH to O_2 in the catalysis.[17] Substantiating this proposition was the finding that the purified enzyme alone could accept two electrons from NADH stoichiometrically in the absence of any electron mediators. The observation of a characteristic free radical signal ($g = 2.002$) in the EPR spectrum obtained anaerobically with a sample of the enzyme and NADH at 8 K may also support the existence of an organic cofactor. Since the putative cofactor should be fully reduced by NADH under these conditions, the radical signal noted may be attributed to electron leakage from the reduced cofactor to residual oxygen in the frozen sample. In fact, the ratio of radical species to protein was estimated to be only 1%. Although the low abundance of this transient radical species was fully anticipated, ascertainment of the significance of such a low level of a radical intermediate must await further scrutiny. Since this enzyme is expected to operate via a single mechanism despite its dual functions as a NADH oxidase and a 3,4-glucoseen reductase, the unique $2e^-/1e^-$ switching capability found for this enzyme provides, for the first time, compelling evidence that it may operate through a radical mechanism.

Thus, the C-3 deoxygenation in the biosynthesis of ascarylose, and possibly the 3,6-dideoxyhexoses in general, may proceed with C–O bond disruption followed by stepwise $1e^-/1e^-$ reduction. The mechanistic revision of the reduction step from a hydride transfer to an electron-transfer process alleviates the fastidious constraint imposed on the guise of the reducing equivalent delivery from E_3 to E_1, since the maximum distance that an electron can move from a donor to an acceptor under physiological conditions is on the order of 10–20 Å.[18] Such long range communication can accommodate a much greater distance between E_3 and E_1 than previously surmised. Furthermore, a radical reduction mechanism could aptly explain the lack of direct hydride transfer from NADPH in the reduction of the 3,4-glucoseen-PMP complex **3** as well. The radical nature of this C-3 deoxygenation process is reminiscent of the well-known sugar deoxygenation reaction catalyzed by ribonucleotide reductase, albeit the mechanisms of these two deoxygenations are fundamentally distinct.[19,20] The answers for the remaining questions await the isolation of homogeneous E_1 and the complete structural characterization of the coenzyme. Work is continuing in these areas, and full details will be reported subsequently.

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(14) The same result was first observed by Rubenstein and Strominger.[2d] With DCPIP as the electron acceptor, this enzyme exhibits a K_m of 53.7 mM for NADH and a V_{max} of 128 nmol·min^{-1} mg^{-1}.

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(16) It is also possible that $O_2^{\bullet-}$ is the obligatory transient intermediate, but only 20% of the $O_2^{\bullet-}$ generated could be trapped by dismutase.

(17) A likely candidate for this organic cofactor is a quinone type coenzyme which is known to have the capability of serving as a $2e^-/1e^-$ switch and, in general, has a UV absorption at ca. 270–300 nm. Furthermore, the NADH oxidase activity can be inactivated by treatment with hydride reducing reagents and phenylhydrazines.

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Polyaza Cavity Shaped Molecules. 15. Stereocontrol in the Formation of Binuclear Complexes

Randolph P. Thummel,* Christophe Hery, Darwin Williamson, and Francois Lefoulon

Department of Chemistry, University of Houston Houston, Texas 77204-5641

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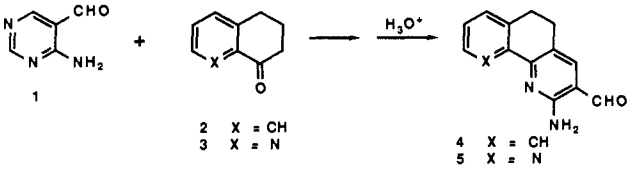
There has been considerable recent interest in the study of macrocycles containing polypyridyl subunits and their metal complexes.[1-8] While cyclic systems are often quite effective in chelating a single metal atom, they do not often lend themselves to the incorporation of more than one metal in a stereocontrolled fashion. As an extension of our work on the conformational properties of monoannelated bipyridine and bis-annelated terpyridine type systems, we herein report the preparation of larger polyaza cavities which show utility in binuclear coordination.

Caluwe and co-workers have demonstrated the usefulness of 4-aminopyrimidine-5-carboxaldehyde (**1**) as a synthon for the



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