^{15}N Nuclear Magnetic Resonance Study of FMN in Flavodoxin

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A FMN derivative was synthesized with all nitrogen atoms of the isoalloxazine ring enriched in the ^{15}N isotope to about 95%. The chemical shifts of the corresponding ^{15}N resonances depend very much on the solvent. On changing from chloroform to water the pyridine-type nitrogen resonances (N(1) and N(5)) of oxidized free FMN shift to higher field by nearly 10ppm, possibly due to stronger hydrogen bonding of water molecules with these nitrogen atoms. The exchange of the N(3)-H with water protons at pH = 5.5 is relatively slow on NMR time scale; the $^{1}J_{^{15}N(3)-^{1}H}$ value can be determined. At higher pH values the corresponding doublet signal coalesces to one single resonance.

In the complex of the oxidized FMN with flavodoxin of P. elsdenii the exchange of the N(3)-H is slow also at high pH values. The $^{15}N(1)$ resonance is shifted to higher field whereas the $^{15}N(5)$ resonance is shifted to lower field.From a comparison of the chemical shift values of free FMN in water and chloroform it seems that the N(5) position in the complex is located in a hydrophobic environment whereas the N(1) position is possibly bound to the enzyme by a hydrogen bond.

For the reduced form 1,5-dihydro FMN dissolved in chloroform a $^{1}J_{^{15}N(5)-^{1}H}$ coupling constant of 88.3Hz was determined. This value is smaller than expected for a more sp^2 hybridized nitrogen (92Hz). Presumably the butterfly vibration around the N(5) - N(10) axis which is assumed to be fast on NMR time scale leads to an average of sp^2 and sp^3 hybridized states of N(5). From the pH dependence of the chemical shift of the 1,5-dihydro FMN $^{15}N(1)$ resonance a pK value of 6.8 can be derived in agreement with corresponding literature values. In the complex with flavodoxin the reduced FMN is bound in the anionic form which is obvious from the chemical shift of the $^{15}N(1)$ resonance. A $^{1}J_{^{15}N(3)-^{1}H}$ and a $^{1}J_{^{15}N(5)-^{1}H}$ value are obtained for the complex. The remarkable high field shift (about 300ppm) of the N(5) resonance upon reduction of FMN in the flavodoxin complex seems to be a clear indication that the N(5) position is the electron acceptor site of flavodoxin and possibly of other corresponding electron transferring enzymes.