Resonance Raman Spectra of Carbon-13- and Nitrogen-15-Labeled Riboflavin Bound to Egg-White Flavoprotein†

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ABSTRACT: The resonance Raman spectra of [2-^{13}C]-, [4a-^{13}C]-, [4-^{13}C]-, [10a-^{13}C]-, [2,4,4a,10a-^{13}C]-, [5-^{15}N]-, [1,3-^{15}N]-, and [1,3,5-^{15}N]riboflavin bound to egg-white proteins were observed for N(3)-H and N(3)-D forms with spontaneous Raman technique by using the 488.0-nm excitation line of an argon ion laser. The fluorescence of riboflavin was quenched by forming a complex with egg-white riboflavin binding protein. The in-plane displacements of the C(2), C(4a), N(1), N(3), and N(5) atoms during each Raman active vibration were calculated from the observed isotopic frequency shifts. The 1252-cm^{-1} mode of the N(3)-H form was found to involve large vibrational displacements of the C(2) and N(3) atoms and to be strongly coupled with the N(3)-H bending mode. This line can be used as an indicator for state of N(3)-H···protein interaction. The 1584-cm^{-1} mode, which is known to be resonance-enhanced upon excitation near the 370-nm absorption band, was accompanied by the displacement of the N(5) atom in particular. The 1355-cm^{-1} mode was most strongly resonance-enhanced by the 450-nm absorption band and involved the displacements of all carbon atoms of ring III. Both lines can be used as structure probes for elucidating the structure of electronically excited states of isoalloxazine.

To elucidate the mechanism of flavoprotein catalysis, physicochemical properties of flavins and their changes upon complex formation with the apoenzyme or upon complex formation of the holoenzyme with substrate should be investigated. Details of the physicochemical nature of flavins have been studied by absorption, fluorescence, and NMR spectroscopies. However, the resonance Raman spectra of flavins have hardly been studied because of strong fluorescence. Only recently, the resonance Raman spectra of flavoproteins have become observable with coherent anti-Stokes Raman scattering (CARS)[1] (Dutta et al., 1977, 1978) or the fluorescence-quenched spontaneous Raman scattering technique (Nishina et al., 1978). In resonance Raman scattering from flavoproteins, the vibrational spectra of the isoalloxazine can be selectively observed without the interference of those of the surrounding amino acid residues of apoprotein. Due to sensitive dependence of the vibrational frequencies upon molecular structure, Raman spectroscopy is potentially a powerful tool for elucidation of the interactions mentioned above. For successful application, however, correct assignments of the resonance Raman lines have to be established on sound experimental basis.

Qualitative assignments of the resonance Raman lines were previously carried out by us on the basis of the observed frequency shifts caused by the replacement of the peripheral groups of the isoalloxazine; some Raman lines were assigned to each of three rings (Nishina et al., 1978). To obtain more detailed assignments, observation of the isotopic frequency shifts for ring atoms and subsequent normal coordinate calculations are indispensable. Therefore, we synthesized ^{13}C- or ^{15}N-labeled riboflavin. Although free riboflavin (RF) is too fluorescent to be subjected to conventional Raman spectroscopy, the fluorescence is completely quenched when RF is bound to egg-white riboflavin binding protein (apo-RBP) (Nishikimi & Kyogoku, 1973). Accordingly, in the present study, we observed the resonance Raman spectra of the isotope-labeled RF in the bound state to apo-RBP, and the vibrational displacement of the C(2), C(4a), N(1), N(3), and N(5) atoms for each of the resonance Raman active modes are reported.

Materials and Methods

Synthesis of [2-^{13}C]-, [4a-^{13}C]-, [4-^{13}C]-, [10a-^{13}C]-, [2,4,4a,10a-^{13}C]-, [5-^{15}N]-, [1,3-^{15}N], and [1,3,5-^{15}N]RF was carried out as reported previously (Yagi et al., 1976a,b), and the more than 98% isotopic substitutions were confirmed by ^{13}C and ^{15}N nuclear magnetic resonance. [4-^{13}C]RF and [10a-^{13}C]RF could not be chemically separated, and their 1:1 mixture was used. Egg-white apo-RBP was prepared according to Rhodes et al. (1959) and purified as described previously (Nishina et al., 1977). RF and apo-RBP were dissolved in 0.1 M sodium phosphate buffer at pH 7.0 (or pD 7.0 for the D_2O solution). Concentrations of RF and apo-RBP were 6.0×10^{-5} and 2.3×10^{-4} M for the H_2O solution and 8.0×10^{-5} and 2.2×10^{-4} M for the D_2O solution; their concentrations were determined spectrophotometrically with the molar extinction coefficients of 1.25×10^4 M^{-1} cm^{-1} at 445 nm for RF (Whitby, 1953) and 4.9×10^4 M^{-1} cm^{-1} at 282 nm for apo-RBP (Nishikimi & Kyogoku, 1973).

Raman scattering was excited at 488.0 nm by an argon ion laser (Spectra Physics, Model 164) and recorded on a JEOL-400D Raman spectrometer equipped with an HTV-R649 photomultiplier. The frequency calibration of the Raman spectrometer was performed with indene (Hendra & Loader, 1968). Through the measurements of the Raman spectra, 300 μL of sample solution was placed in a thermostated longitudinal cell kept at 15 ± 2 °C, and excitation light with sufficiently low power (60 mW) was irradiated from the bottom of the cell.

Results

The resonance Raman spectra of nonlabeled, [2-^{13}C]-, [4a-^{13}C]-, [5-^{15}N]-, and [1,3-^{15}N]RF bound to RBP are shown in Figures 1 and 2, where the 1:1 mixture of [4-^{13}C]- and [10a-^{13}C]RF is also included. Atomic and ring numberings

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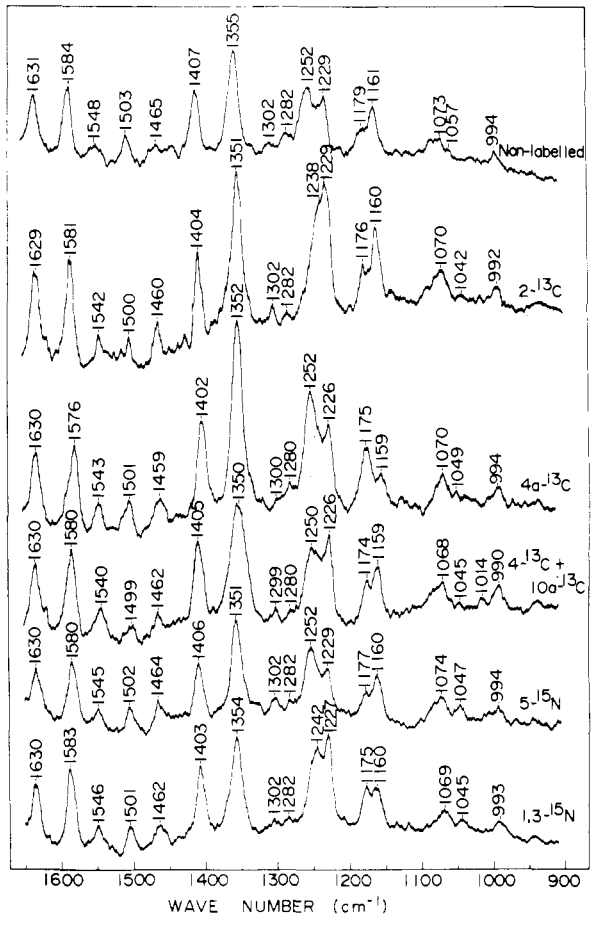

FIGURE 1: Resonance Raman spectra in the higher frequency region of the N(3)-H form of the labeled RF in the bound state to RBP.

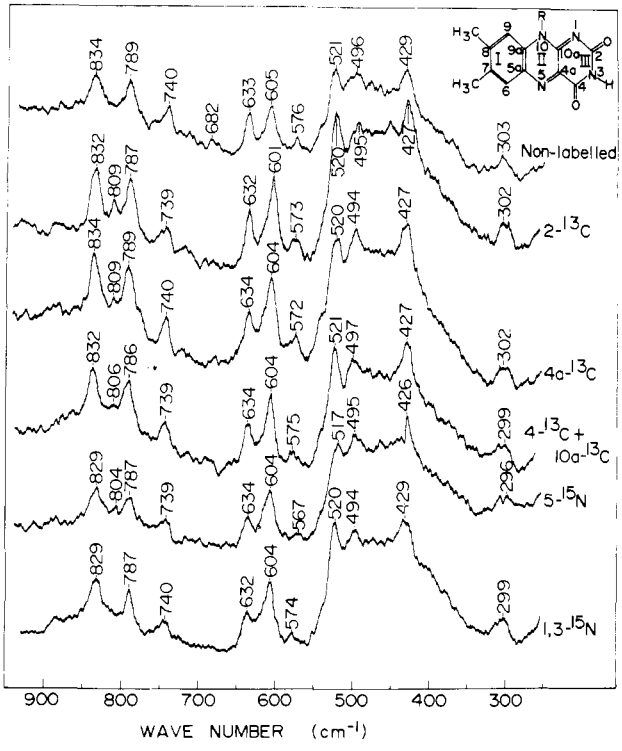

FIGURE 2: Resonance Raman spectra in the lower frequency region of the N(3)-H form of the labeled RF in the bound state to RBP. The inset indicates the numbering of atoms and rings of the isoalloxazine.

of isoalloxazine are indicated in an inset of Figure 2. The spectra of [2,4,4a,10a-^{13}C]- and [1,3,5-^{15}N]RF (not shown) were substantially identical with those in Figures 1 and 2, though the largest isotopic frequency shifts among the labeled samples were observed. Relative intensities of Raman lines, 1252/1229, 1179/1161, and 633/605 cm^{-1}, varied with isotopic substitutions. The frequency shifts [$\Delta\nu = \nu$(nonlabeled) $- \nu$(isotope labeled)] of Raman lines are listed in Table I. It was noted that the frequency shift for [1,3,5-^{15}N]RF was close to the sum of the frequency shifts for [5-^{15}N]- and [1,3-^{15}N]RF. This implies that the normal coordinates of nonlabeled RF are altered little by isotopic substitutions and mass effects are additive. The mixture of [4-^{13}C]- and [10a-^{13}C]RF gives a few broad Raman lines for which the isotopic frequency shifts are different between 4-^{13}C and 10a-^{13}C substitutions but are not resolved.

Table I: Observed Isotopic Frequency Shifts for the Resonance Raman Lines of Riboflavin of the N-H Form (cm^{-1})

	^{13}C			^{15}N		
RF	2-	4a-	2,4,4a,-10a-	5-	1,3-	1,3,5-
1631	2	1	2	1	1	2
1584	3	8	13	4	1	5
1548	6	5	21	3	2	4
1503	3	2	7	1	2	4
1465	5	6	8	1	3	6
1407	3	5	12	1	4	6
1355	4	3	15	0	1	1
1302	0	2	5	0	0	2
1282	0	2		0	0	0
1252	14	0	17	0	10	10
1229	0	3	4	0	2	2
1179	3	4	8	2	4	6
1161	1	2	5	1	1	2
1073	3	3	3	−1	4	2
994	2	0	4	0	1	3
834	2	0	2	5	5	10
789	2	0	4	2	2	6
740	1	0	2	1	0	2
633	1	−1	0	−1	1	2
605	4	1	4	1	1	4
576	3	4	5	9	2	9
521	1	1	2	4	1	7
496	1	2		1	2	4
429	2	2	2	3	0	6

The large isotopic frequency shifts of the 1252-cm^{-1} line upon 2-^{13}C substitution (1238 cm^{-1}) or 1,3-^{15}N substitution (1242 cm^{-1}) were noticed. This suggests that the 1252-cm^{-1} mode involves vibrational displacements of the C(2) and N(3) atoms in particular and thus the C(2)–N(3) stretching motion. If it is true, this line corresponds to amide III of the cis amide group [O=C(2)–N(3)-H] and should be sensitive to deuterium substitution of the amide group (Miyazawa, 1960).

The N(3) hydrogen of RF is replaced by deuterium in D_2O solution. The resonance Raman spectra of labeled RF of the N(3)-D form are shown in Figure 3, where the spectrum of nonlabeled RF of the N(3)-H form is represented with a broken line. As expected, the 1252-cm^{-1} line of nonlabeled RF of the N(3)-H form disappears upon deuteration and a new line appears at 1295 cm^{-1}. The 1161-cm^{-1} line of nonlabeled RF of the N(3)-H form also shows a large frequency shift to 1147 cm^{-1} upon N(3)-deuteration. The 1138-cm^{-1} line of nonlabeled RF of the N(3)-D form would not be due to an isotope-shifted line of nonlabeled RF of the N(3)-H form at 1161 cm^{-1}, since a prominent line around 1135 cm^{-1} is observed only for the N(3)-D form in the cases of uracil derivatives (Lord & Thomas, 1967).

The isotopic frequency shifts of RF of the N(3)-D form are listed in Table II. The 1295-cm^{-1} line of nonlabeled RF of the N(3)-D form shows a large frequency shift upon 1,3-^{15}N

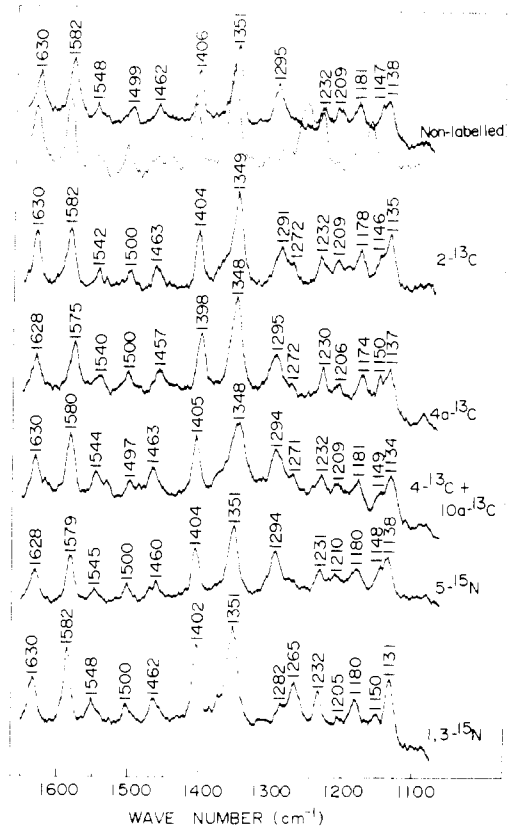

FIGURE 3: Resonance Raman spectra of the N(3)-D form of the labeled RF in the bound state to RBP.

Table II: Observed Isotopic Frequency Shifts for the Resonance Raman Lines of Labeled Riboflavin of the N-D Form (cm^{-1})

nonlabeled		^{13}C			^{15}N		
N-H[a]	N-D	2-	4a-	2,4,4a,-10a-	5-	1,3-	1,3,5-
1631	1630	0	2	3	2	0	2
1584	1582	0	7	11	3	0	4
1548	1548	6	8	24	3	0	7
1503	1499	−1	−1	2	−1	−1	−1
1465	1462	−1	5	4	2	0	6
1407	1406	2	8	12	2	4	6
1355	1351	2	3	15	0	0	0
1252	1295	4	0	10	1	30	30
1229	1232	0	2	4	1	0	2
1179	1181	3	7	12	1	1	2
1161	1147	1	−3	1	−1	−3	−3
	1138	3	1	12	0	7	6
994	990	2	1	2	2	−2	1
834	832	1	0	3	5	7	12
789	772	2	1	2	3	2	6
740	744	1	0	0	2	0	1
633	629	0	2	2	1	1	2
605	602	2	1	6	3	1	4
521	522	1	0	2	2	0	4
496	474		−1	−2	−8		
429	427	1	−1	2	0	1	5
300	300	1	0	1	−1	−1	0

[a] The N-H form. The frequencies are the same as those in the first column of Table I.

and 1,3,5-^{15}N substitutions but a quite small shift upon 2-^{13}C substitution in contrast with that of the N(3)-H form. This suggests that a significant change has occurred in the 1252-cm^{-1} mode of the isoalloxazine upon N(3)-deuteration.

The 1355-cm^{-1} line, which was markedly intensified in resonance with the 450-nm absorption band, showed a frequency shift upon the substitution of carbon atoms but not of nitrogen atoms in both the N(3)-H and N(3)-D forms. On the other hand, the Raman line at 1584 cm^{-1}, which was the only line resonated with the 370-nm band (Tsuboi, 1976), shifted upon 4a-^{13}C and 5-^{15}N substitution in both the N(3)-H and N(3)-D forms.

Table III: Vibrational Displacements (Å) of Atoms for Riboflavin of the N-H Form

mode (cm^{-1})	C(2)	C(4a)	N(5)	N(1), N(3)
1631	0.005	0.004	0.004	0.003
1584	0.007	0.011	0.008	0.003
1548	0.010	0.009	0.007	0.004
1503	0.007	0.006	0.004	0.004
1465	0.009	0.010	0.004	0.005
1407	0.007	0.010	0.004	0.006
1355	0.009	0.008	0	0.003
1252	0.018	0	0	0.011
1229	0	0.009	0	0.005
1179	0.009	0.010	0.007	0.007
1161	0.005	0.007	0.005	0.004
834	0.010	0	0.016	0.012
789	0.011	0	0.011	0.008
740	0.008	0	0.008	0
633	0.010	0	0	0.007
605	0.021	0.010	0.010	0.007
576	0.019	0.022	0.033	0.011
521	0.013	0.013	0.025	0.009
496	0.013	0.019	0.013	0.013

Discussion

The visible absorption band of flavin near 450 nm, with which the present Raman scattering was resonated, is due to the π–π^* transition of the isoalloxazine, and its transition dipole is in the molecular plane (Sun et al., 1972). Therefore, the resonance enhancement of Raman lines is expected only for the modes in which atoms are displaced within the molecular plane during the vibrations. By assumption that $X_{\kappa a}$ is the vibrational displacement of the κth atom due to the ath mode, it is correlated with the frequency shift of the ath mode ($\Delta\nu_a$) for isotopic substitution of the κth atom as represented by eq 1 (Miyazawa, 1964), where ν_a^0 and ν_a are the frequencies

$$\langle X_{\kappa a}^2 \rangle = \frac{h(\nu_a^0 + \nu_a)}{8\pi^2 \nu_a^3 c} \frac{\Delta\nu_a}{\Delta m_\kappa} \frac{m_\kappa}{m_\kappa^0} \frac{[1 + \exp(-h\nu_a/kT)]}{[1 - \exp(-h\nu_a/kT)]} \quad (1)$$

of the ath mode of nonlabeled and isotope-labeled molecules, respectively, m_κ^0 and m_κ are the masses of nonlabeled and isotope atoms, respectively, and Δm_κ is a mass difference ($= m_\kappa - m_\kappa^0$). The vibrational displacements at 293 cm^{-1} derived as a root of $\langle X_{\kappa a}^2 \rangle$ were calculated for the N(3)-H form of RF as listed in Table III. Although eq 1 is valid only for a monosubstituted molecule, approximate quantities of the vibrational displacements of N(1) and N(3) atoms were estimated as large as a root of $\langle X_{\kappa a}^2 \rangle/2$ derived from $\Delta\nu_a$ for [1,3-^{15}N]RF (Table III). If only one of the pair of the labeled atoms was involved in a given mode, the true displacement would be equal to $2^{1/2}$ times the displacement listed in Table III.

It is noticed in Table III that motions of atoms in different rings are appreciably involved in a single vibrational mode. This prominent delocalization of vibrational energy is characteristic of such a multiring system. As vibrational frequencies become lower, the displacements become larger. For accurate determination of the isotopic frequency shifts in the lower frequency region, the measurements of Raman spectra at lower temperature would be necessary. However, as the temperature decreased, fluorescence increased seriously and observation of Raman spectra was impossible even at 5 °C.

The 1631-cm^{-1} line of RF showed quite small frequency shifts upon isotopic substitutions in D_2O as well as in H_2O solution even for the mixture of [4-^{13}C]- and [10a-^{13}C]RF. If this line were the C=O stretching mode, the line should show a large frequency shift upon C(2) or C(4) isotopic substitution. Furthermore, for the O=C(2)–N(3)-H linkage in cis conformation, the C=O stretching frequency should be affected by deuterium substitution, but, actually, the 1631-cm^{-1} line of RF is unaltered. This feature is also true for FAD in H_2O (1635 cm^{-1}) and in D_2O (1634 cm^{-1}) (Dutta et al., 1977). Since the 1631-cm^{-1} line of RF is most sensitively affected by 7,8-dichlorination (Kitagawa et al., 1978), the line is presumably associated with a ring I vibration. Thus, the reported small frequency change (5 cm^{-1}) of the 1635-cm^{-1} line of FAD upon incorporation into glucose oxidase (Dutta et al., 1977) might be attributed to a change of the overall π electronic state of ring I.

The transition dipole for the 370-nm band was calculated to be nearly along C(7) → C(2) (Sun et al., 1972). In the Raman spectrum of FAD resonated with the 370-nm band, only the 1584-cm^{-1} line was observed (Tsuboi, 1976). The 1584-cm^{-1} mode is accompanied by large displacements of C(4a) and N(5) in both the N(3)-H and N(3)-D forms. Since the resonance enhancement of Raman lines generally occurs in the modes which approximately reproduce the molecular geometry of the electronically excited state during the vibration (Hirakawa & Tsuboi, 1975), the 370-nm transition might involve appreciable stretch of the C(4a)–N(5) bond. In contrast, the 1355-cm^{-1} line is markedly intensified in resonance with the 450-nm band and is accompanied by large displacements of the substituted carbon atoms but not of the substituted nitrogen atoms in both the N(3)-H and N(3)-D forms. This suggests that this mode is associated with the stretching vibrations of the C(4a)–C(10a)–N(1)–C(2) linkage. By consideration of the appearance of intense Raman lines of ring III modes upon excitation at 488.0 nm, the 450-nm transition might be associated more significantly with ring III than with rings I and II.

Drastic changes of the relative intensity, 1179/1161 cm^{-1}, upon isotopic substitutions are interesting. Apparently, the higher frequency mode is accompanied by larger displacement of all the ring III atoms than is the lower one (Table III). This region of the Raman spectrum also showed complicated alteration upon replacement of the N(3) substitution (Nishina et al., 1978). Accordingly, these lines might be practically useful as a structural probe of flavin coenzyme interacting with apoenzyme or substrate, although we may refrain from detailed discussion on them until normal coordinate calculations would be completed.

The 1252-cm^{-1} line of RF of the N(3)-H form involves the atomic displacements of ca. 0.015 Å of C(2) and 0.011 Å of N(3), exhibiting distinct changes in frequency and intensity upon 2-^{13}C and 1,3-^{15}N substitutions. Upon N(3)-deuteration, ca. 40 cm^{-1} of high-frequency shift was observed, in good agreement with the results on FAD (Dutta et al., 1978), and the displacement of N(3) became larger (0.02 Å) but that of C(2) became smaller (0.009 Å). Previously, a similar high-frequency shift was observed on N(3)-methylation and N(3)-carboxymethylation (Nishina et al., 1978) and was interpreted as follows. The 1252-cm^{-1} mode involves mainly C(2)–N(3)–C(4) stretching vibrations and is shifted to lower frequency from its intrinsic frequency due to the vibrational coupling with the N(3)-H bending mode, but it comes back to the intrinsic frequency (~1295 cm^{-1}) when the vibrational coupling with the N(3)-H bending mode is removed by the replacement of the N(3) hydrogen with carbon. The N(3)-H bending mode is located presumably around 1420 cm^{-1} as it was for uracil derivatives (Miles et al., 1973) and, therefore, the N(3)-D bending mode of RF of the N(3)-D form would lie below 1250 cm^{-1}. This change of the N(3)-H bending frequency upon N(3)-deuteration would result in an appreciable change in the C(2)–N(3)–C(4) stretching mode. Accordingly, the changes of displacements of C(2) and N(3) atoms upon N(3)-deuteration observed in this study are consistent with the interpretation proposed previously.

When flavin–protein interaction is related to a contact of the N(3) proton with an appropriate proton acceptor of protein, the stronger N(3)-H···protein hydrogen bond generally results in higher frequency of the N(3)-H bending mode. Then the 1252-cm^{-1} vibrations would be less coupled with the N(3)-H bending mode and as a result would show a high-frequency shift. In this sense the 1252-cm^{-1} line may serve as an indicator of a state of N(3)-H···protein interaction. The C(2)–N(3)–C(4) stretching mode was observed at 1260 cm^{-1} for free FAD but was too weak to be detected for glucose oxidase (Dutta et al., 1978). This difference between free FAD and glucose oxidase indicates appreciable difference of C(2)–N(3)–C(4) stretching modes between them, presumably reflecting difference in N(3)-H···X hydrogen bondings. For the N(3)-D form of FAD, on the other hand, the mode was observed at 1297 cm^{-1} for free FAD and at 1291 cm^{-1} for glucose oxidase (Dutta et al., 1977). This difference between the N(3)-H and the N(3)-D forms is consistent with our interpretation.

Previously, Nishikimi & Kyogoku (1973) studied the interaction between riboflavin and RBP and concluded that RF is placed in a less polar environment with some aromatic groups of amino acid residues close to the N(3) position. This conclusion is consistent with the results of solvent perturbation difference spectroscopy by Blankenhorn (1978), who suggested a stacking interaction of tyrosine with the isoalloxazine. This stacking interaction presumably results in the quenching fluorescence, enabling the observation of spontaneous Raman scattering. Generally, this type of interaction appreciably affects the intensity but much less the frequency of Raman lines.

A ^{13}C NMR study by Yagi et al. (1976a) showed that the C(4)-carbonyl group participates in the interaction with RBP. The present Raman study did not give additional information on the mode of interaction of the carbonyl group because of the absence of the definite Raman line assignable to the carbonyl stretching mode. When the data of visible absorption spectra and ^{13}C NMR are combined, it can be said that ring III of the isoalloxazine is related to the interaction with RBP. As discussed above, the 1252-cm^{-1} line was observed for RF–RBP but missed in the CARS spectrum of glucose oxidase (Dutta et al., 1977). This may be explained by assuming the difference in the mode of the N(3)-H···X bond between RF–RBP and glucose oxidase. Blankenhorn (1978) pointed out that the 7,8 positions of isoalloxazine are buried in RF–RBP in contrast with the case of flavodoxin (Watenpaugh et al., 1973). If such difference also exists between egg RBP and glucose oxidase, it may explain the difference in the behavior of the 1252-cm^{-1} line.

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Base Interactions in the Triplet States of NAD^+ and NADH†

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ABSTRACT: We report here the phosphorescence spectra, 77 K lifetimes, triplet state zero-field splittings (zfs), and optical detection of magnetic resonance (ODMR) linewidths of the coenzyme nicotinamide adenine dinucleotide. The triplet state parameters of both the oxidized and reduced coenzyme (NAD^+ and NADH, respectively) were compared with those of the model compounds ADP, ADP-ribose, and 1-methyl-3-carbamidopyridinium chloride, and it was found that the major phosphorescence of NAD^+ and NADH is due to the adenine base. In aqueous ethylene glycol glasses, we found that the adenine triplet state in the coenzyme was strongly perturbed. There were large shifts in the $|D - E|$ and $|D + E|$ transitions, considerable broadening of the zf resonances, and changes in the sign of the zf spin polarizations. Furthermore the zfs of neither NAD^+ nor NADH were additive ($|D - E| + |2E| \neq |D + E|$), in contrast to those of ADP and ADP-ribose. The discrepancy was approximately six times larger for NAD^+ (117 MHz) than for NADH (19 MHz); the discrepancy is expected to be not larger than about 10 MHz based on the precision of the experimental data. In nonaqueous propylene glycol, the zfs of NAD^+ and NADH were additive and similar to those of ADP-ribose. Consideration of all the triplet state data, including 77 K phosphorescence lifetimes and ODMR linewidths, suggests that, in frozen *aqueous* solution, 95–100% of the coenzyme molecules are folded, and more than one conformation is stable for NAD^+, whereas one conformation appears to predominate for NADH. In neutral propylene glycol, in the absence of water, the intramolecular association is weak.

Luminescence studies on nicotinamide adenine dinucleotide, an important coenzyme in biological oxidation–reduction reactions, have long focused on the fluorescence of the reduced form of the dinucleotide (NADH),[1] since the oxidized form (NAD^+), by comparison, is essentially nonfluorescent. The observed fluorescence of NADH is due to the dihydronicotinamide base; the fluorescence yield of adenine by itself is negligible.[2] Fluorescence excitation spectra of NADH in aqueous solutions show bands at 260 and 340 nm. Upon enzymatic cleavage of the diphosphate bridge joining the two nucleotides, the 260-nm peak in the excitation spectrum disappears and has therefore been attributed to energy transfer from adenine to the dehydronicotinamide base in a stacked conformation (Weber, 1957; Scott et al., 1970).

Supportive evidence for intramolecular association of the dinucleotide bases has been obtained from proton NMR studies. Early experiments at 60 MHz provided a picture in which the coenzyme exists in a rapid equilibrium between folded and unfolded conformers (Jardetsky & Wade-Jardetsky, 1966; Sarma et al., 1968). At 220 MHz the two C4 protons of the dihydronicotinamide ring in NADH were resolved. Their nonequivalence indicated two folded forms (Sarma &

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[1] Abbreviations used: NAD^+, nicotinamide adenine dinucleotide; NADH, dihydronicotinamide adenine dinucleotide; EGW, ethylene glycol–water; AMP, adenosine 5′-monophosphate; ADP, adenosine 5′-diphosphate; ADP-ribose, adenosine 5′-diphosphoribose; MeNCl, 1-methyl-3-carbamidopyridinium chloride; NMR, nuclear magnetic resonance; ODMR, optically detected magnetic resonance; zfs, zero-field splittings.

[2] Eastman & Rosa (1968) measured the fluorescence quantum yield of adenine in 30/70 (v/v) water–ethylene glycol as a function of temperature and obtained a value of less than 0.0005 at 298 K.

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