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RAMAN SPECTROSCOPY OF LIVER ALCOHOL DEHYDROGENASE[#]

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SUMMARY: We report the Raman spectrum of liver alcohol dehydrogenase in solution. The enzyme's secondary structure as determined from an examination of the Raman bands is slightly different than that found in crystals by X-ray diffraction.

Horse liver alcohol dehydrogenase (EC-1.1.1.1.), LADH, catalyzes the oxidation of various primary and secondary alcohols to the corresponding aldehydes (1,2) along with the reduction of the coenzyme nicotinamide adenine dinucleotide, NAD^+. Eklund et al. (3) reported a detailed three-dimensional structure at 2.4Å resolution with X-ray diffraction of crystalline LADH. Additional results have been obtained with X-ray diffraction and solution spectroscopy of LADH complexed with coenzyme or its various analogs and its ternary complexes with different substrates or inhibitors (4-6). The recent work of Abdallah et al. (7) provides an elegant example of such a study.

Raman spectroscopy has proved to be useful in providing information on the conformation of biological molecules (8-13). In particular, it can provide information on samples in solution as well as in solid or crystalline states. For example, Yu et al. (10) demonstrated that there is a conformational difference between insulin crystals and insulin in solution as determined by Raman spectroscopy. Kint and Tomimatsu (13) showed that this technique is sensitive in detecting small changes in protein conformation. We report here a non-resonance Raman spectroscopic study of LADH in solution.

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METHODS AND MATERIALS

LADH was purchased from Boehringer Mannheim Co. (Indianapolis, IN). The enzyme suspension was centrifuged at 4°C at 6000Xg for 20 minutes. The pellet was re-dissolved in 0.05M pyrophosphate buffer (pH = 9.60) and then centrifuged again at 4°C at 6000Xg for 10 minutes to remove denatured protein. The supernatant was then dialyzed overnight against 1 litre of 0.1M pyrophosphate buffer (pH = 9.60) and then concentrated using a collodion bag vacuum concentrator (A. H. Thomas, Philadelphia, Pa.). The activity was measured by the method of Dalziel (14). No denaturation of the enzyme was detected during the experiment. The enzyme was put into a fluorescence cuvette in a thermobath maintained at 8°C. Raman spectra were obtained by an OMA system: the spectrometer consists of a pair of gratings set up to give no net dispersion and a selection of three dispersive gratings (TRIPLEMATE, Spex Industries, Metuchen, N.J.); an Argon ion laser (Spectra Physics, Mountain View, Ca.); a water cooled photodiode array (model 1420, Princeton Applied Research, Princeton, N.J.) controlled by a model 1208 controller (PAR) which is interfaced to a mini-computer (LSI-11, Digital Equipment Corp., New York, N.Y.). The spectral lines were calibrated against known Raman lines of toluene. A deuterated LADH sample was prepared by dilution with a D_2O-containing buffer solution, which was allowed to stand overnight (15) and was then concentrated by a Centricon microconcentrator (AMICON, Danver, Ma.). The dilution and concentration was repeated prior to the spectroscopic measurement.

RESULTS AND DISCUSSION

Figure 1 shows a typical Raman spectrum of LADH at pH = 9.60. Most of the peaks can be attributed either to the protein backbone or to amino acid "R-groups". Assignments were made by comparisons with Raman spectra of other proteins and amino acids in solution (8,12). Table 1 lists the preliminary assignments of most peaks to various parts of the enzyme.

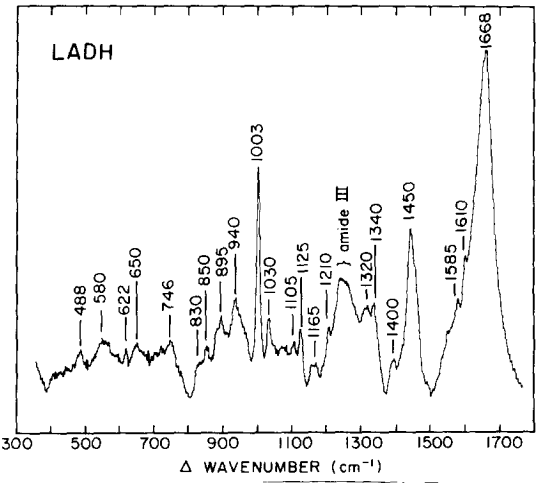

Figure 1: Typical Raman spectrum of liver alcohol dehydrogenase in .1M pyrophosphate buffered solution (pH=9.6). Sample was maintained at 8°C. Laser line was 488.0 nm with incident power of 110 mw. Dispersive grating was 1200 cm^{-1} with slit width of 100 μm, giving a final resolution of 6 cm^{-1}.

Table 1: Principal peak frequencies in the Raman spectrum of liver alcohol dehydrogenase (Fig. 1)

Peak Frequency (cm^{-1})	Tentative Assignment
488	
580	
622	Phe ring mode
650	
746	
830^{sh}	Tyr
850	Tyr
895	
940	
1003	Phe
1030	Phe
1105	
1125	C-C stretch in hydrocarbon
1165	Phe, Tyr
1210	Phe, Tyr
1230^{sh}	Amide III: β-sheet
1245	Amide III: random coil
1265^{sh}	Amide III: α-helix
1320	
1340	γ-CH_2
1400	Asp, Glu: COO^-
1450	δ-CH_2
1585	Phe, Arg
1610	Phe, Tyr
1668	Amide I

Aromatic residues are known to give strong Raman lines. LADH has only 2 tryptophan (Trp) and 4 tyrosine (Tyr) residues. Thus contribution from these amino acid groups is expected to be small. For example, the 850 cm^{-1} and 831 cm^{-1} bands commonly associated with tyrosine (16) are only barely visible. However, there are 18 phenylalanine (Phe) residues in LADH, and they are responsible for the prominent sharp peak at 1003 cm^{-1} and for other smaller bands. Peaks from aromatic amino acid residues are known to be insensitive to the conformational state of the protein (8).

There are two prominent bands due to the peptide backbone of the enzyme. The amide I band appears at 1668 cm^{-1} and the amide III bands are at 1230^{sh}, 1245, and 1265^{sh} cm^{-1}. Raman spectral analysis of the enzyme in D_2O shows that the amide III bands shift towards 900-1000 cm^{-1} as expected. A small broad structure remains at 1200-1300 cm^{-1} which may be due to other parts of LADH or to incomplete proton/deuterium exchange. Both amide vibrations are known to be sensitive to the secondary structure of the proteins (9, 11, 17). Typical ranges for these bands are: for amide I, 1645-1660 cm^{-1} for α helix,

1665-1680 cm^{-1} for β sheet and 1660-1670 cm^{-1} for unstructured random coil; for amide III, 1265-1300 cm^{-1} for α helix, 1230-1240 cm^{-1} for β sheet and 1240-1260 cm^{-1} for random coil. Various methods have been used to calculate the percentages of each type of structure (18-21). Lippert et al. (18) defined the intensities at three frequencies which are indicative of three types of secondary structure: 1240 cm^{-1} (amide III) for β-sheets; 1632 cm^{-1} (amide I') for α helix and 1660 cm^{-1} (amide I') for random coil. Typical intensities for each type of structure at these frequencies were obtained from model polypeptides and proteins with known structure. The sum of the intensities for the different types of structures scaled down by its corresponding percentages gives the observed Raman intensity. Percentages of each type of structure were calculated by solving the simultaneous equations. Following this procedure, we found that LADH in solution contains 21% α helices, 35% β-pleated sheets, and 43% of unstructured random coil. These percentages are close to the structure obtained by X-ray crystallography (29% helix, 34% β-pleated sheet and 37% random coil; Eklund et al., 1976). Following a simplified method of Williams et al., (20) which uses a calculation of structure based only on the amide I bands, a low percentage of α helix (18%) was also obtained. The small difference in helical structure as determined by the X-ray and Raman techniques may be indicative of small conformational changes that occur upon dissolving the crystals into this buffer solution.

In addition, we have been unable to detect any pH dependence of the Raman spectrum of LADH from pH 7.0 to 9.6. A pH dependent conformational change in the LADH structure defined by a pK of 9.8 was postulated previously (22). At pH 9.6, there exists approximately 40% of the deprotonated form whereas none of this form is present at pH 7.0. The Raman spectra of LADH at these two pH's are identical to within less than 3%. Measurements at pH values higher than 9.6 were precluded because LADH denatures under these conditions. Nevertheless, we conclude that over the pH range of 7.0 - 9.6, only small changes in the secondary (or tertiary) structure of LADH occurs and that these changes are not detectable by Raman spectroscopy.

In conclusion, we present the non-resonance Raman spectrum of LADH. The calculated secondary structure in solution differs slightly with that obtained by X-ray diffraction on crystals.

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