UV Resonance Raman Elucidation of the DNA-Binding Domain of the Cancer Tumor Suppressor p53 Protein

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The DNA-binding core domain (residues 94-312) of the cancer tumor suppressor p53 protein has 1 Trp, 8 Tyr, 9 His and 1 Zn atom tetrahedrally coordinated to 3 Cys and 1 His. The UVRR spectra of the p53 core domain (p53cd) are obtained with excitation at λ_{ex}=229 nm to study the structure, denaturation state with changes in pH and to evaluate the state of the UV active amino acid residues of the protein and its metal (Zn^{2+}) coordination by H-D exchange.

The intensity of the W7 doublet at pH 7 indicates that the Trp environment is hydrophobic. As the pH is lowered to pH 5, the decrease in the intensity of W18 and W16 bands, confirms an increase in the hydrophilicity of the Trp environment.

On deuteration (pD 7 to pD 3), the Tyr hydrogen bonding marker bands were downshifted by 10 cm^{-1} for Y8b and 3 cm^{-1} for Y8a. The state of hydrogen bonding is changed on deuteration.

The His marker bands of the deuterated form showed the τ tautomer band, **ν5a** (1353 cm^{-1}) at pD 7. The appearance of this band is attributed to His ligand being coordinated to the metal ion. The band diminishes with lower pD, while the band at 1408 cm^{-1} grew in the p53cd. The appearance of the 1408 cm^{-1} band is also observed for the Zn(II)-containing culture expressing the p53cd protein. The Zn bound to His is therefore broken at lower pH.

The spectral features of Tyr and Trp are conserved as the pH was decreased to pH 3. This suggest that denaturation of p53cd was not observed.

In conclusion, the results of the UVRR study have provided structural information of the p53cd in its aqueous state. On deuteration, the following were found: (a) the Tyr is hydrogen bonded, (b) the protonation of the His side chain is detected, and (c) the cleavage of the Zn-His bond at lower pD is confirmed. The hydrophobic environment of Trp is revealed. Furthermore, the conservation of the spectral features of Tyr and Trp implies that p53cd resists against denaturation at lower pH.