Ultraviolet resonance Raman study of streptavidin binding of biotin and 2-iminobiotin

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Introduction
Streptavidin is a bacterial, tetrameric protein that binds biotin extremely tightly and has a structural motif similar to that of avidin. This exceptionally strong binding affinity has been the object of fundamental studies of protein/substrate interactions and the basis of numerous biotechnological applications. The binding of biotin to avidin and streptavidin has three major components; hydrophobic interactions especially with tryptophan, hydrophilic interactions via an extensive complimentary hydrogen bonding network and the closure of a flexible loop around the substrate. Despite the similarities between streptavidin and avidin there are important differences between these proteins. Avidin is a glycoprotein, containing one disulphide bridge and two methionine residues, whereas streptavidin is non-glycosylated and has no sulphur containing residues. The binding of biotin to avidin and streptavidin has been investigated by a number of spectroscopic techniques, including ultra-violet (UV) absorption, circular dichroism (CD) and fluorescence spectroscopy. The UVRR technique has the advantage in many biological applications of providing specific enhancement from the Trp and Tyr residues and therefore avidin and streptavidin, which have multiple aromatic residues in the binding site, represent excellent systems for study in the development of this relatively new technique.

Streptavidin binding of biotin and 2-iminobiotin
Streptavidin solutions were prepared in phosphate buffered saline at pH 7.5 to which 5 mM KNO₃ was added to serve as an internal intensity standard for Raman difference spectroscopy. Solid biotin, or 2-iminobiotin was added to approximately 10 times excess to the streptavidin solution, gently shaken for 5 minutes and kept on ice for 1 hour. Raman spectra were acquired using a Renishaw micro-Raman system 1000. Accurate difference spectra were produced using the 1048 cm⁻¹ band of the potassium nitrate as an internal reference standard.

Figure 1 shows the UVRR spectra of streptavidin and the streptavidin/biotin complex in solution at pH 7.5 and the difference spectrum. The spectra are dominated by the contributions from the eight Trp residues in streptavidin, with moderate contributions from the six Tyr residues and from the amide I band at 1662 cm⁻¹. Upon binding of biotin a number of spectral changes occur to the streptavidin UVRR spectrum that are clearly visible in the difference spectrum and that can be attributed to both Tyr and Trp vibrational bands.

In the 2-iminobiotin molecule the carbonyl group of biotin is replaced by a guanidino group and the effect of this structural difference on the UVRR difference spectrum of the 2-
iminobiotin/streptavidin system is shown in Figure 2. The most striking contrast between the two difference spectra in Figures 1 and 2 are the contributions that can be assigned to Tyr 43 which dominate the difference spectrum in Figure 2. The change in the local hydrogen bonding network together with the difference in local dipole-dipole interactions of biotin and 2-iminobiotin with streptavidin account for the difference in the Tyr 43 contributions to the UVRR difference spectra.

**Figure 2** UVRR spectra at pH 7.5 of streptavidin (spectrum a), streptavidin–2-iminobiotin complex (spectrum b), and the difference spectrum (spectrum b-a). The intensities of spectra a and b are normalized to the internal standard NO₃⁻ peak at 1048 cm⁻¹, and the difference spectrum is scaled by a factor of 2 for clarity.

**Conclusions**
The UVRR technique is particularly well suited to study the streptavidin biotin and 2-iminobiotin complexes as the binding site Trp and Tyr residues are resonantly enhanced at 244 nm. The hydrophobic interactions of biotin and 2-iminobiotin with the Trp residues were found to be similar, however, significant differences in the environment of Tyr 43 were observed. UVRR spectroscopy clearly resolves both the Trp and the Tyr contributions to the binding of biotin and 2-iminobiotin to streptavidin and highlights the differences in the Tyr 43 environment between these complexes.

**References**
