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Cooperative Allosterism in Avidin and Streptavidin Upon Biotin Binding as Observed by Changes in Intrinsic Fluorescence

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Abstract

Avidin is a homotetrameric protein known for its high binding affinity and specificity for biotin which gives it great functionality in many biotech applications. Prior research on the bacterial analog, streptavidin found cooperative allosterism exhibited in the binding process. This research was aimed at investigating whether this same cooperative allosterism accompanies the binding of biotin to avidin. This work measured intrinsic tryptophan fluorescence as biotin was titrated into avidin. A blueshift in the wavelength of maximum fluorescence as well as quenching of the overall fluorescence intensity, similar to the streptavidin was observed. The saturation point for 335 nm emission (tryptophans in more hydrophobic environments) was lower than the 4:1 stoichiometric ratio exhibited by the 350 nm emission (tryptophans in more hydrophilic environments). This suggest biotin binding to avidin exemplifies cooperative allosterism similar to that of streptavidin. Additional fluorescence based parameters were also examined and will be described relative to those for streptavidin as well.

Background

Avidin (AV) and streptavidin (SA) are very similar proteins in many respects. Both proteins have a very strong and specific binding affinity for biotin with a dissociation constant being in the range of 10^{-15}. Both proteins can also bind with up to 4 biotin molecules thanks to their tetrameric structure. One significant amino acid difference between AV and SA is the number of tryptophans in each monomer. SA has 6 tryptophans while AV only has 4. Tryptophans give these proteins intrinsic fluorescence which varies based on the local chemical environment[1,2].

The AV/SA-Biotin system is used in a large number of biotech applications. The interaction between biotin and AV is the strongest known noncovalent interaction between protein and ligand. Binding of biotin affects the structures of SA and AV, which in turn influences the fluorescence of their tryptophans. Tryptophans can emit light at different wavelengths with the highest fluorescence intensity wavelength affected by external environment. The wavelength of fluorescence emission depends on the local chemical environment, with those in more polar environments emitting near 350 nm and those in more hydrophobic (non-polar) environments emitting near 335 nm[2,3]. Biotin binding to SA changes the environment of its tryptophans causing a significant blue shift while also reducing or quenching the total fluorescence intensity.

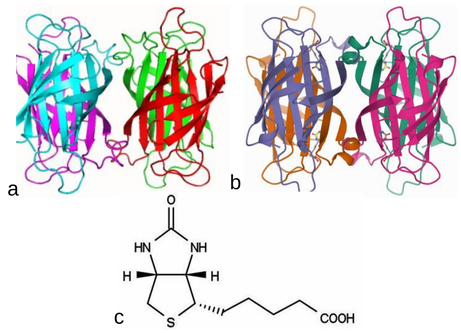

Figure 1: Structure of a) AV, b) SA, and c) biotin

Upon biotin binding to one SA monomer, a structural change happens to that monomer. At the same time, similar structural changes happen to an adjacent monomer without changing the intrinsic binding affinity of biotin to that monomer [3]. This mechanism is based on cooperative allosterism. Figure 2 presents the proposed model of biotin binding to SA based on cooperative allosterism. The aim of this experiment is to examine whether or not AV also follows this model based on cooperative allosterism.

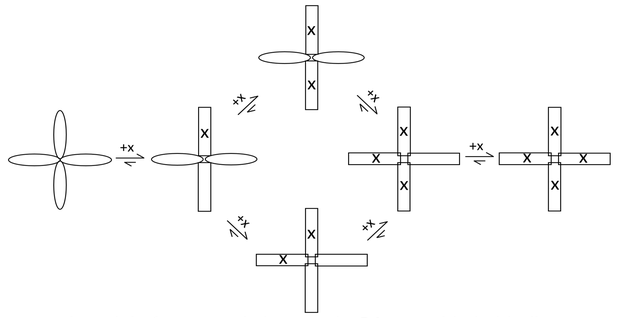

Figure 2: Proposed model of structural changes in SA upon biotin binding

Methods

A Photon Technology International QuantaMaster Dual-Emission spectrofluorimeter was used to measure fluorescence as biotin was titrated into a solution of avidin and buffer. Excitation was at 280 nm. Photomultiplier tube voltages were 1000-1063 V. A 2-minute equilibration period followed each addition of protein or ligand. Additionally, the samples were constantly stirred. Between each reading, the excitation shutter was closed to avoid photobleaching. The emission maxima of all trials was kept below the signal saturation point of the instrument at 3.7×10^6. Starting with 1.8 mL of 10 mM TEA buffer, 5-30 µL of protein was added. 5 µL aliquots of 3.88 µM biotin solution were titrated into the protein solution.

Results

Emission scans of avidin as biotin was added is shown in Figure 2. Emission data collected at 335 nm and 350 nm shows the breakpoint of avidin in Figure 3. Table 1 shows the full width at half maximum for AV and SA.

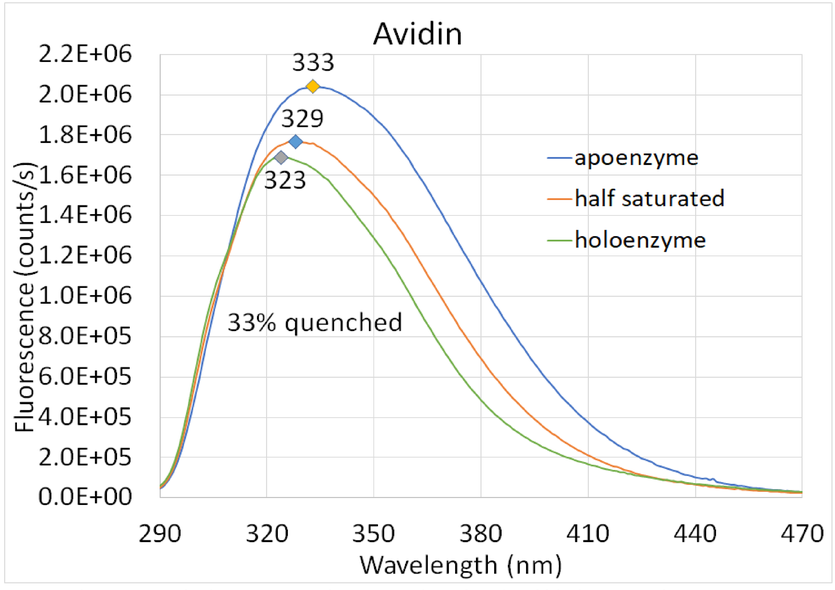

Figure 3: Emission spectra of AV at various levels of biotin saturation

	Full Width at Half Maximum
Avidin	3.87
Streptavidin	3.83[3]

Table 1: Full width at half maximum data for AV and SA

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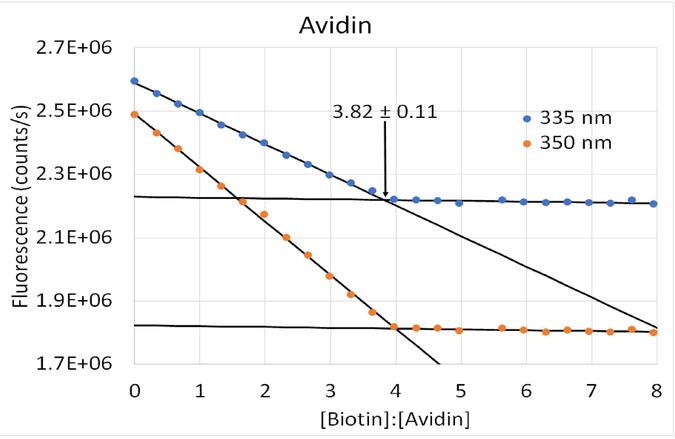

Figure 4: Fluorescence titration of biotin into AV, monitoring emission at 335 nm and 350 nm

Discussion

As the apoenzyme, AV had a maximum fluorescence wavelength of 333 nm. As the holoenzyme, the maximum fluorescence wavelength was 323 nm. Previously published work shows a similar blue shift with SA as the maximum fluorescence wavelength goes from 335 nm to 327 nm as biotin is added[3]. As for the quenching of fluorescence intensity, the total integrated intensity decreased by 33% for AV which is similar to the 30% decrease seen in SA total integrated intensity[3]. As seen in figure 2, there is a break in the emission of both wavelengths near the 4:1 stoichiometric ratio of biotin to AV. The break point of the 350 nm emission was used as the 4:1 stoichiometric ratio as it is the established standard measure of ligand saturation in both the biotin/AV and biotin/SA systems. Using this breakpoint, the 335 nm emission was consistently found to saturate earlier. Replicate measurement show the 335 nm emission saturating at 3.82 ± 0.11 when excited at 280 nm. This is statistically the same as data collected with SA which found the 335 nm emission saturation point to be 3.76 ± 0.18[3].

Conclusions

These results and previous work suggest that biotin binding to AV exemplifies cooperative allosterism to a similar extent as SA. Biotin binding to one AV monomer causes similar structural changes to an adjacent monomer without changing the intrinsic binding affinity of biotin to that monomer. These finding with AV suggests the model proposed to explain biotin binding to SA based on cooperative allosterism can work with AV as well. As a dimer of dimers, biotin binding to one monomer of AV induces a structural change to both that monomer as well as the one across from it. The next biotin molecule can bind to any of the three-remaining monomers, but it is statistically more likely that it will bind to one of the monomers that is still in the native state. This means all 4 AV monomers will have undergone significant biotin induced structural changes within the first 2 binding events. If the second biotin molecule binds to the monomer across from the first, by the third binding event, the most significant structural changes of AV will occur. Regardless of the location of the second and third biotin binding events, the significant structural changes of AV caused by biotin binding will occur before the fourth biotin molecule binds. This mechanism is an explanation as to why AV reaches its breakpoint before the 4:1 stoichiometric ratio at 335 nm.

Future Directions

An extension of this work would be to see how the salt concentration of the TEA buffer could affect the results. Work with salt buffers has started with some preliminary results showing a higher concentration of salt buffer can raise the saturation point of avidin at 335 nm to approximately 4.

References

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