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COOPERATIVE ALLOSTERISM IN AVIDIN AND STREPTAVIDIN UPON BIOTIN BINDING AS OBSERVED BY CHANGES IN INTRINSIC FLUORESCENCE

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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 suggests 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.

Introduction

The study of the avidin-biotin and streptavidin-biotin is ongoing and has been used in research and technology for a dating back to the 1970s (*1*). Avidin and streptavidin are very similar proteins in many respects despite coming from different sources. Avidin comes from many different sources including hen egg whites while streptavidin comes from the soil bacteria *Streptomyces avidini*. Both proteins have a very high and specific binding affinity for biotin with a dissociation constant (K_d) being in the range of 10^{-15} for both proteins. Both proteins bind with up to 4 biotin molecules thanks to its homotetrameric structure with each monomer consisting of an 8 stranded antiparallel beta barrel with extended loops that connect the beta strands. In addition, neither protein has any disulfide bonds due to a lack of cysteines in both proteins which

is unusual for proteins so stable. This stability can be attributed to the extensive intermolecular forces and interactions between the 4 subunits of an avidin or streptavidin molecule (*2*). This stability is increased upon biotin binding (*1*).

Even though these proteins are very similar, there are some differences in structure, especially in the loops that connect the beta sheets as well as in the amino acid sequence of the proteins. One particularly significant amino acid difference between avidin and streptavidin is the number of tryptophans in each monomer. Streptavidin has 6 tryptophans (*3*) while avidin only has 4 (*1*). The tryptophans in these proteins give them intrinsic fluorescence which was measured in this work. The fluorescence of the tryptophans varies based on the local chemical environment (*1*, *4*). Streptavidin is also larger to avidin at 159 amino acids compared to the 128 amino acids that make up avidin (*1*). It has a 125-127 amino acid core that is very similar to avidin with a quartet of identical tryptophan residues that are involved in biotin binding (*1*). These characteristics make both systems good at visualizing target molecules.

There are several advantages to using these systems in this way thanks to its versatility and high binding affinity which is why it is applied so often in biotechnology applications like ELISA (enzyme linked immunosorbent assay), immunohistochemistry, western, northern, and southern blotting, immunoprecipitation, affinity purification, and EMSA (electrophoretic mobility shift assay) (*3)*.

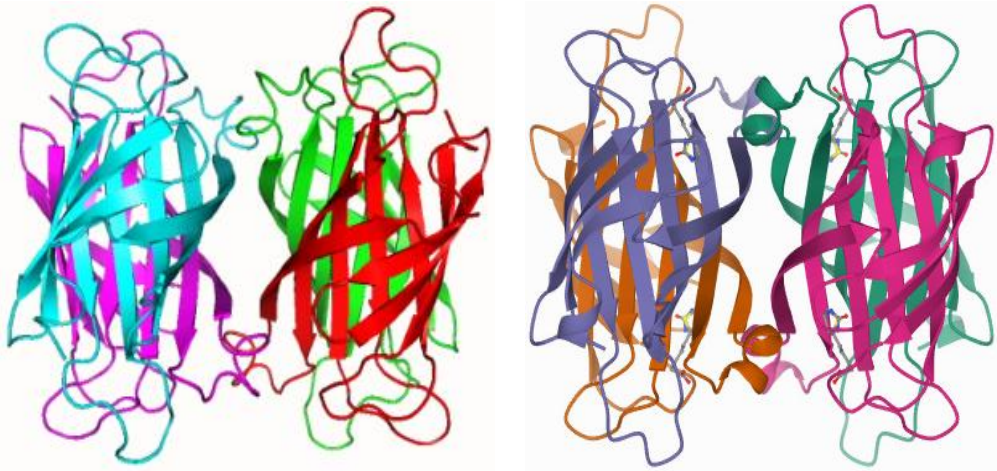

Figure 1 Structure of avidin (left) and streptavidin (right) molecules (Reprinted from https://www.weizmann.ac.il/Biomolecular_Sciences/Bayer/research-activities/avidin-biotin-system [avidin] and https://www.rcsb.org/structure/2IZI [streptavidin])

The specific ligand that binds to these proteins is biotin, which is also known as vitamin H or vitamin B7. Biotin is almost a perfect fit for the biotin binding pockets of avidin and streptavidin. It is such an ideal shape that when it is not present, solvent molecules in the binding site resemble the shape of a biotin molecule (*5*). Biotin is commonly used in biotech applications in conjunction with avidin or streptavidin thanks to its high binding affinity and high specificity to both of those proteins. The interaction between biotin and avidin is the strongest known noncovalent interaction between protein and ligand. Once biotin binds with avidin or streptavidin, it is unaffected by extreme conditions like pH, temperature, and organic solvents which makes the biotin/avidin and biotin/streptavidin systems extremely useful in purifying or detecting proteins of interest.

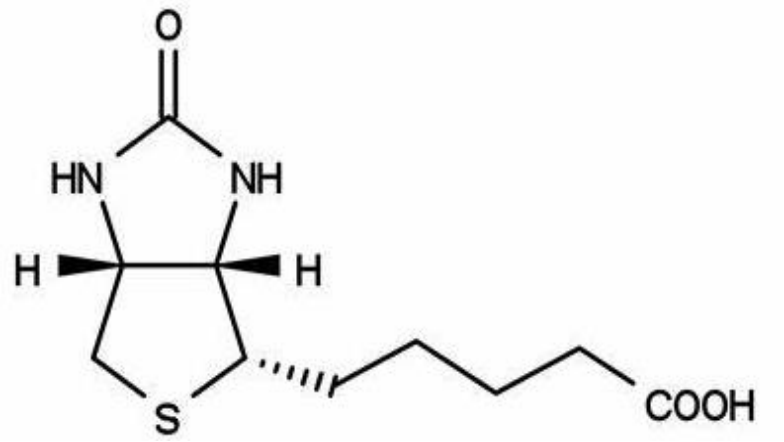

Figure 2 Structure of biotin. (Reprinted from https://pubchem.ncbi.nlm.nih.gov/compound/171548#section=2D-Structure)

Binding of biotin affects the intrinsic fluorescence of the tryptophans in avidin and streptavidin. Fluorescence is the light emitted by a substance that has absorbed light at a different wavelength. Tryptophan is one of 3 amino acids that can do it intrinsically with the other two being tyrosine and phenylalanine. The tryptophans in avidin absorb light in the 280-290 nm range, which is in the ultraviolet portion of the electromagnetic spectrum. The tryptophans then emit light at different wavelengths, but the fluorescence of the tryptophan residues is highly dependent on local chemical environment. As the polarity of the environment decreased, the wavelength at which the fluorescence intensity is the greatest blue shifts to slightly lower wavelengths in a polar solvent tryptophans will fluoresce primarily at 350 nm while tryptophans in a nonpolar solvent will fluoresce closer to 335 nm (*4*, *6*). Previous research has found that biotin binding to streptavidin changes the environment of its tryptophans by changing its structure causing a significant blue shift of its maximum fluorescence intensity (*7*). In addition to shifting the wavelength of the maximum intensity, biotin binding also reduces or quenches the fluorescence intensity of the tryptophans (*6*, *7*). A similar result is found with avidin in this experiment. In this experiment, the mechanism in which biotin binds to avidin was investigated and compared to previous data with streptavidin due to their similarities. For both avidin and

streptavidin, upon biotin binding to one 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. These results suggested a avidin follows the same mechanism of biotin binding as streptavidin based on cooperative allosterism. The binding of the first and second biotin are the most likely to cause the most significant structural changes, but these changes are mainly based on where the biotins bind (*7*).

Results

Emission scans of avidin as biotin was added is shown in Figure 3. Emission data collected at 335 nm and 350 nm shows the breakpoint of avidin in Figure 4. Table 1 shows the full width at half maximum for avidin and streptavidin.

Emission scans: titration of biotin into avidin

Emission data was collected from 290 nm to 470 nm. As the apoenzyme, avidin had a maximum fluorescence wavelength of 333 nm. At half saturation, the maximum fluorescence wavelength was 329 nm. As the holoenzyme, the maximum fluorescence wavelength was 323 nm. At half saturation, the maximum fluorescence wavelength was 329 nm. As biotin was added, the wavelength with the maximum fluorescence blueshifts (Figure 3). Previously published work demonstrated a similar blueshift with streptavidin as the maximum fluorescence wavelength goes from 335 nm to 327 nm as biotin is added. (*6*) As for the quenching of fluorescence intensity, the total integrated intensity decreased by 33% for avidin which is similar to the 30% decrease seen in streptavidin total integrated intensity (*6*) as the holoenzyme.

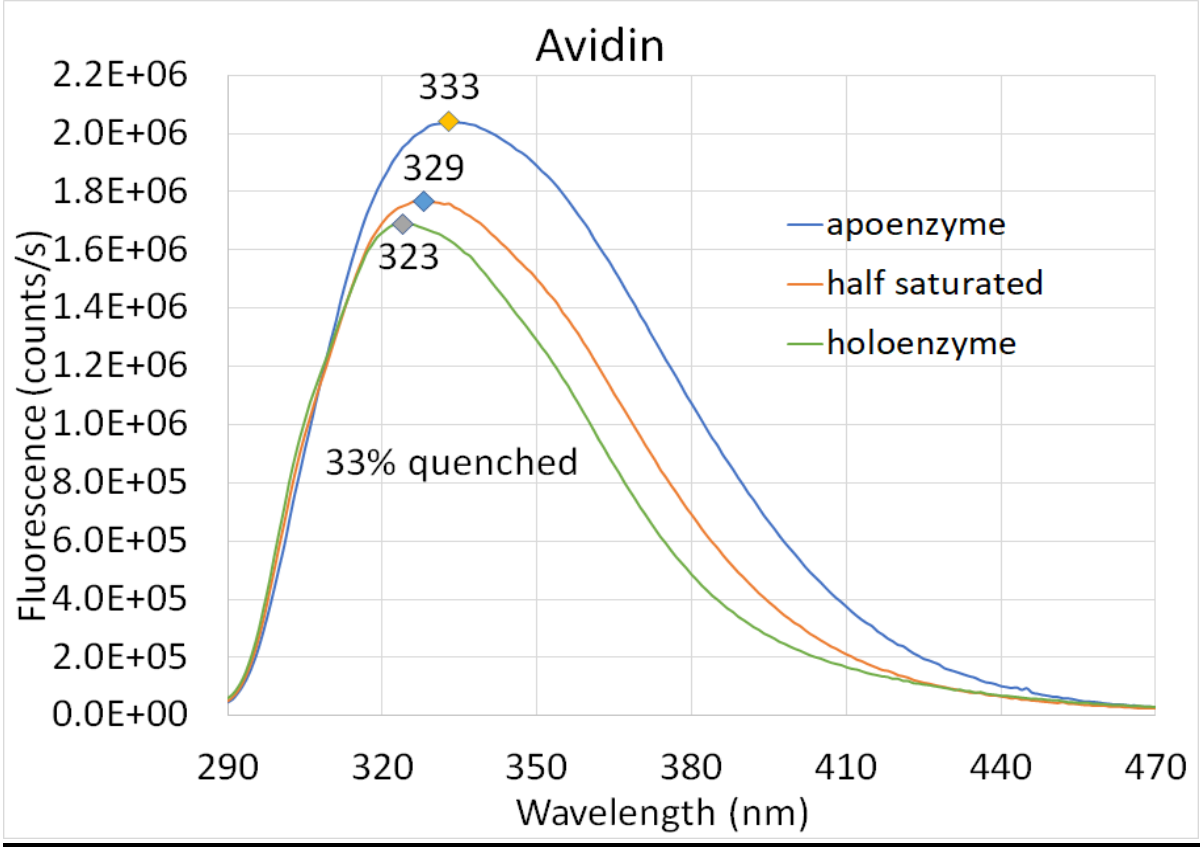

Figure 3 Emission Spectra of avidin at various levels of biotin saturation.

Biotin binding also an effect on the full width at half maximum (FWHM) of the fluorescence of both avidin and streptavidin. As the apoenzyme, the FWHM was __ for avidin while it was 57 nm for streptavidin (*7*). The FWHM for the holoenzymes of these avidin and streptavidin were __ and 50 nm, respectively (*7*). This indicates a __ nm reduction in the FWHM for avidin and a 7 nm reduction for streptavidin. The decrease in FWHM for both avidin and streptavidin indicate a wider distribution of tryptophan residues in hydrophilic and hydrophobic environments, but upon ligand binding this distribution narrows to more residues in more hydrophobic environments. The structural changes to avidin and streptavidin caused by biotin binding resulted in their tryptophans shifting to more common shared environment (Table 1).

Table 1 Full width at half maximum for avidin and streptavidin.

	[biotin]:[protein]	Full Width at Half Maximum	
		Apoenzyme	**Holoenzyme**
Avidin	3.87		
Streptavidin (*7*)	3.83	57 nm	50 nm

Emission data collected at 335 nm and 350 nm

Since the previous results were so similar with avidin compared to streptavidin, this suggested avidin was behaving similarly to streptavidin. To get a more detailed look at what is happing to the fluorescence of avidin, emission data was only collected at 335 nm and 350 nm. This data would determine the [biotin]:[avidin] ratio at which each fluorescence signal saturates which could then be compared to the [biotin]:[streptavidin] ratio. There is a break in the emission of both wavelengths near the 4:1 stoichiometric ratio of biotin to avidin. 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/avidin and biotin/streptavidin systems.

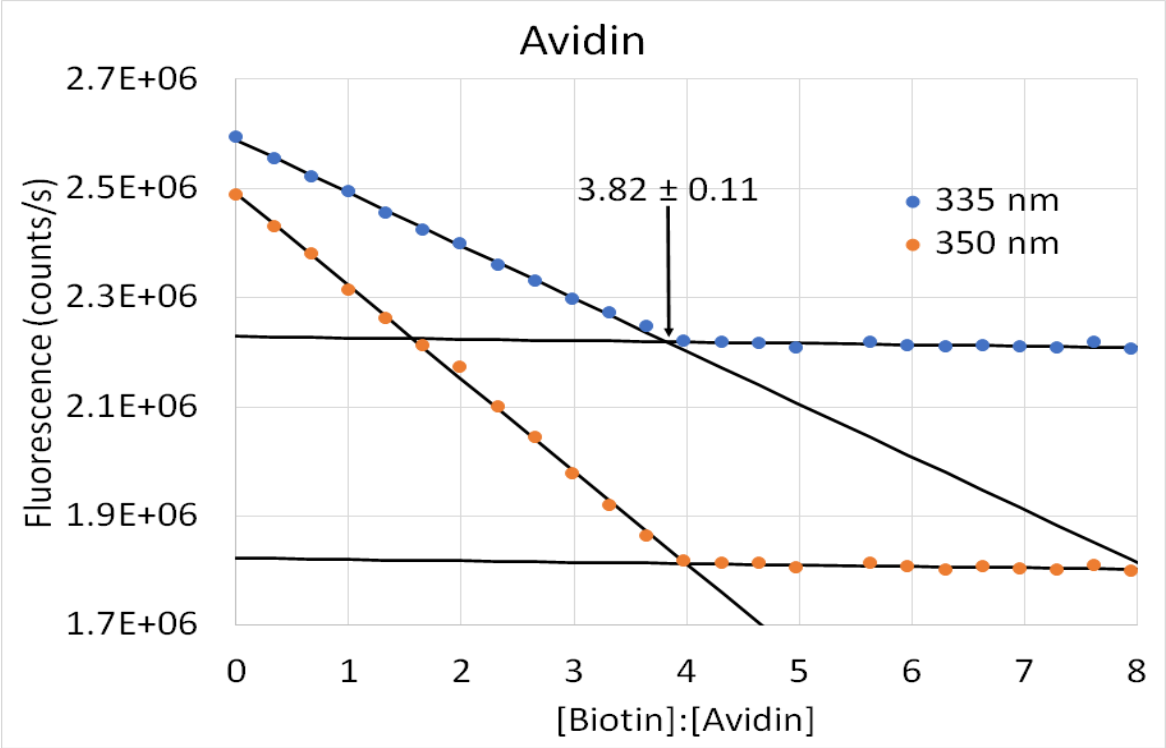

Figure 4 Fluorescence titration of biotin into avidin, monitoring emission at 335 nm and 350 nm.

Using this breakpoint, the 335 nm emission was consistently found to saturate earlier. Replicate measurement show the 335 nm emission of avidin saturating at 3.82 ± 0.11 when excited at 280 nm (Figure 4). An ANOVA test found this saturation point to be statistically the same as data collected with streptavidin. The saturation point of streptavidin at 335 nm was found to be 3.76 ± 0.183 (*7*).

Discussion

These results and previous work suggest that biotin binding to avidin exemplifies so called, 'cooperative allosterism' similar to streptavidin. Cooperative allosterism combines the ideas of cooperativity and allosterism. Traditional cooperativity is the binding of ligand to one binding site affects the binding strength of subsequent ligand binding events. The change to the neighboring subunit results in the affinity for the binding of a second ligand molecule to be altered either positively or negatively. Hemoglobin is the typical example when thinking about

positive cooperativity as the binding of one oxygen molecule increase the binding affinity of other oxygen molecules to the hemoglobin. Allosterism is a change in the binding affinity of a ligand that is caused by binding of another ligand or substrate away from the active site which typically is accompanied by some kind of structural changes to the protein.

Combining these concepts in this context results in biotin binding to one avidin monomer causing similar structural changes to an adjacent monomer without changing the intrinsic binding affinity of biotin to that monomer. The findings presented here for avidin suggest the model proposed to explain biotin binding to streptavidin based on cooperative allosterism applies to avidin as well. As a dimer of dimers, biotin binding to one monomer of avidin 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 four avidin monomers will have undergone significant biotin induced structural changes within the first two 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 avidin will occur. Regardless of the location of the second and third biotin binding events, the significant structural changes of avidin caused by biotin binding will occur before the fourth biotin molecule binds. This mechanism is an explanation as to why avidin saturates before the 4:1 stoichiometric ratio at 335

nm.

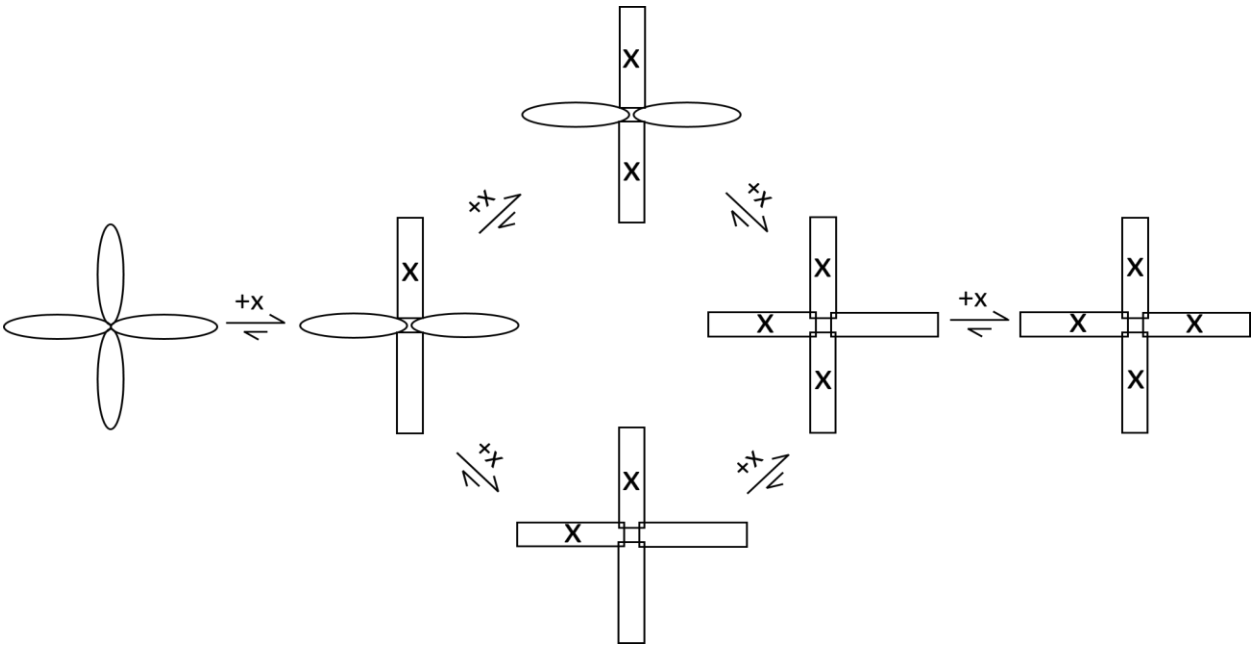

Figure 5 Proposed model of structural changes in streptavidin and avidin upon biotin binding. (7)

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. In addition, more statistical analysis can be done with some of the data that has already been collected.

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 (magnetically). Between each reading, the excitation shutter was closed to avoid photobleaching. The emission maxima of all trials were 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 approximately 3.88 µM biotin solution were titrated into the protein solution.

Acknowledgements

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