Novel cholesterol binding peptides: Design, synthesis and *in vitro* biochemical evaluation

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Introduction

One of the major contributing factors to cardiovascular disease and stroke are high levels of serum cholesterol, bound with Low Density Lipoprotein (LDL-C). This condition results in plaque formation on the inside walls of arteries, leading to blockages in blood flow.

Research has shown that physiological proteins, such as *Synuclein (Syn)* and *Cholesterol Recognition/Interaction Amino Acid Consensus (CRAC)*, bind with cholesterol to reduce plaque formation.

Potent cholesterol-binding peptides (CBP) were developed from these proteins by solid phase peptide chemistry. Their *in vitro* affinity towards cholesterol was determined using fluorescence spectroscopy and SELDI-tof mass spectrometry.

Hypothesis & Objectives

The three CBPs will bind with cholesterol, resulting in quenching of fluorescence, and this binding will lower LDL-C levels in the blood.

The aim was to examine the fluorescence properties of cholesterol and CBPs in order to study the potential interactions with LDL-C. This may help find potential therapeutic applications for cholesterol management.

Table 1. List of the designed cholesterol binding peptides based on the amino acid sequences of known physiological proteins or consensus sequence

Peptide Name	Cholesterol Binding Protein	Amino Acid Sequence	Calculated Average MW (Da)	Observed MW
CPB-1	hSynuclein (66-78)	^{66}WGGAVVTGVTAVA^{78}	1186.36	1186.2
CPB-2	LS-CDC Protein (473-484)	^{473} ECTGLAWEWWRT ^{484}	1537.71	1537.6
CPB-3	CRAC Sequence	TVLNYYVW	1057.21	1078.9 (+Na)

Methodology

- Three cholesterol binding peptides were synthesized from cholesterol-dependent cytolysins (CDC), cholesterol recognition/ interaction amino acid consensus (CRAC) and Synuclein proteins by solid phase peptide chemistry, as well as a control peptide (WEPFASGKTSESGELHGLTT-$CONH_2$) known not to bind with cholesterol.
- They were characterized using SELDI-tof mass spectrometry.
- The excitation and emission fluorescence spectra for all peptides were also determined using a spectrofluorometer (Spectramax, Gemini XS).
- These were performed in aqueous sample solutions at various concentrations (total 25 µL volume) in a black 96-well plate (Dynetac). The concentration of all peptides was 1 mg/mL.
- Increasing amounts of cholesterol solution in isoamyl alcohol were added to the CBPs in aqueous solution with and without incubation.
- The *in vitro* binding and affinity potentials of the peptides towards cholesterol were determined based on the results of the fluorescence study, with excitation wavelength fixed at 280nm.

Results

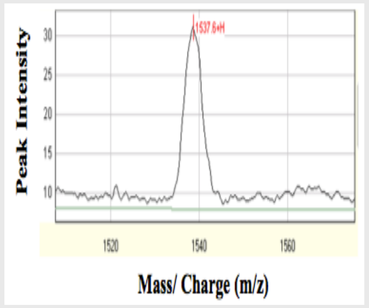

Figure 1. SELDI-Tof mass spectrum (expanded) of crude CBP-1

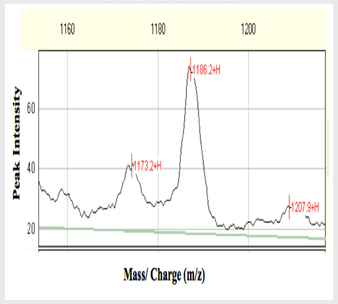

Figure 2. SELDI-Tof mass spectrum (expanded) of crude CBP-2

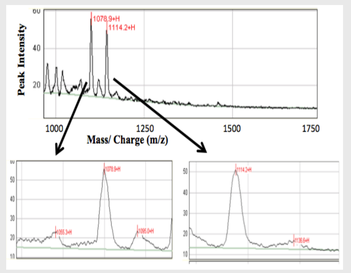

Figure 3. SELDI-Tof mass spectrum of crude CBP-3. Below: Expanded in two regions

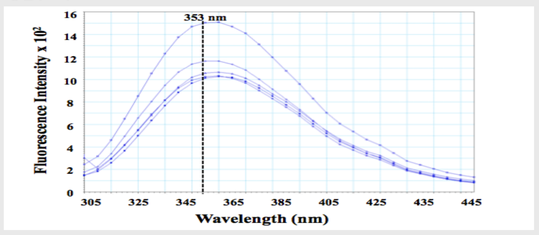

Figure 4. Emission fluorescence spectrum of CBP-2 in aqueous solution at various concentrations with excitation wavelength fixed at 280 nm

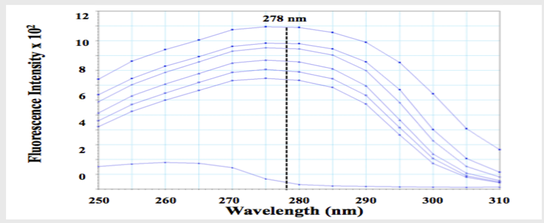

Figure 5. Excitation fluorescence spectrum of peptide CBP-2 in aqueous solution at various concentrations with emission wavelength fixed at 360 nm

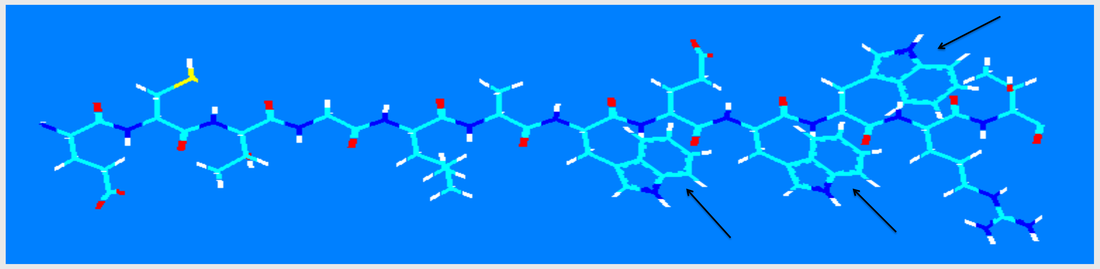

Figure 6. Model of CBP-2 (ECTGLA**W**E**WW**RT). The arrows point to the three tryptophans (W) in the sequence.

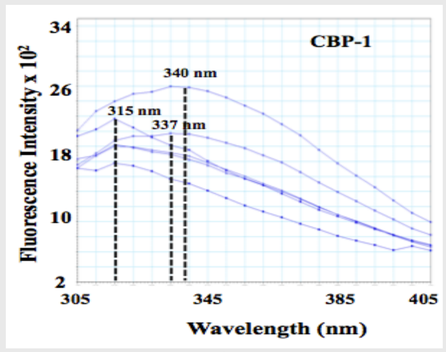

Figure 7. Emission fluorescence spectrum of aqueous CBP-1 (1mg/mL) in cholesterol/ isoamyl alcohol solution

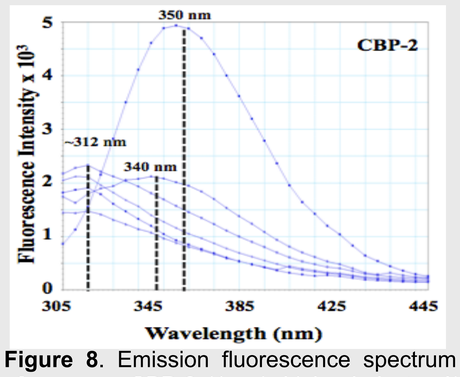

Figure 8. Emission fluorescence spectrum of aqueous CBP-2 (1mg/mL) in cholesterol/ isoamyl alcohol solution

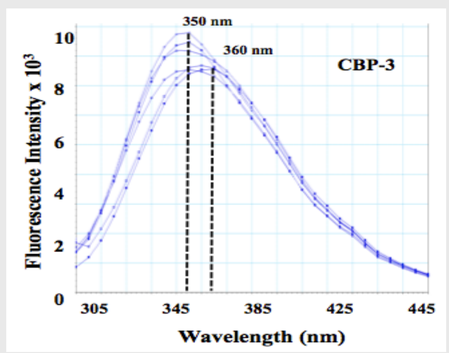

Figure 9. Emission fluorescence spectrum of aqueous CBP-3 (1mg/mL) in cholesterol/ isoamyl alcohol solution

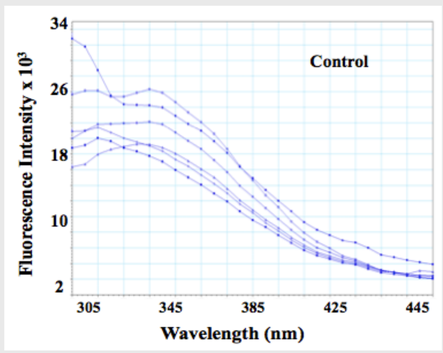

Figure 10. Emission fluorescence spectrum of control peptide in aqueous solution (1mg/ mL) in cholesterol/isoamyl alcohol solution

Discussion

- The gradual shifting of the peak maxima and quenching of fluorescence intensity with increasing amounts of cholesterol suggest binding of cholesterol with the peptide.
- Of the three peptides, CBP-2 binds very strongly, followed by CBP-1 and CBP-3, as reflected by the fluorescence levels decreasing in Figures 7-9.
- Since CBP-2 has three fluorescent tryptophan residues rather than one, it also had the overall highest fluorescence intensity.
- The fluorescence intensity of CBP-1, CBP-2, and CBP-3 were diminished by 58%, 35%, and 13% respectively.
- Some shifting in the wavelength may also be observed. CBP-3 has a marginal shift but quenching is common to all.
- The control peptide has a slight but negligible peak, otherwise indicating it did not bind with cholesterol.
- This demonstrates that the domains from the physiological proteins are necessary in the binding of CBPs with cholesterol.

Table 2. Changes in fluorescence intensity after addition of cholesterol

Peptide	Change in Fluorescence Intensity (%)
CPB-1	58.0
CPB-2	34.6
CPB-3	13.3

Conclusion

All three CBPs interacted with cholesterol, as indicated by the suppression of fluorescence intensity. CBP-2 had excitation and emission wavelengths of 353nm and 278nm respectively, and also had the greatest affinity for cholesterol when compared with the other two peptides. Having a high affinity for cholesterol suggests that these peptides may be able to bind and block LDL-cholesterol interactions. This may potentially lower levels in the blood, which would be useful for cholesterol management in diseases such as hypercholesterolemia and other CVDs. Future directions for this project include using a mass spectrometer or more robust methods, such as cell culture work. As well, circular dichroism spectrum could be used to determine any structural changes in the peptides upon binding with cholesterol.

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