

Purpose

The circular dichroism spectrum of AmilCP was analyzed to determine the major secondary structure features of AmilCP. This can be used to determine the purity of AmilCP in future purified samples of AmilCP along with providing insight on the specific interactions that contribute to the protein's stability.

Methods

AmilCP () transformed into DH5- α was grown in an LB liquid culture for 3 days. The resulting culture was centrifuged and resuspended in ddH₂O. The sample was pelleted again and resuspended in lysis buffer. After centrifugation, the supernatant was purified using a Sephadex G-100 Superfine column. The sample was purified in two runs, the first to separate the larger molecules and lysis buffer from the protein and a second run to further purify the protein. As a chromoprotein, fractions were preserved based on their blue color, indicating the presence of AmilCP. The fractions were then analyzed by circular dichroism to determine optical rotation of circularly polarized light as it interacts with the secondary structures within the protein.

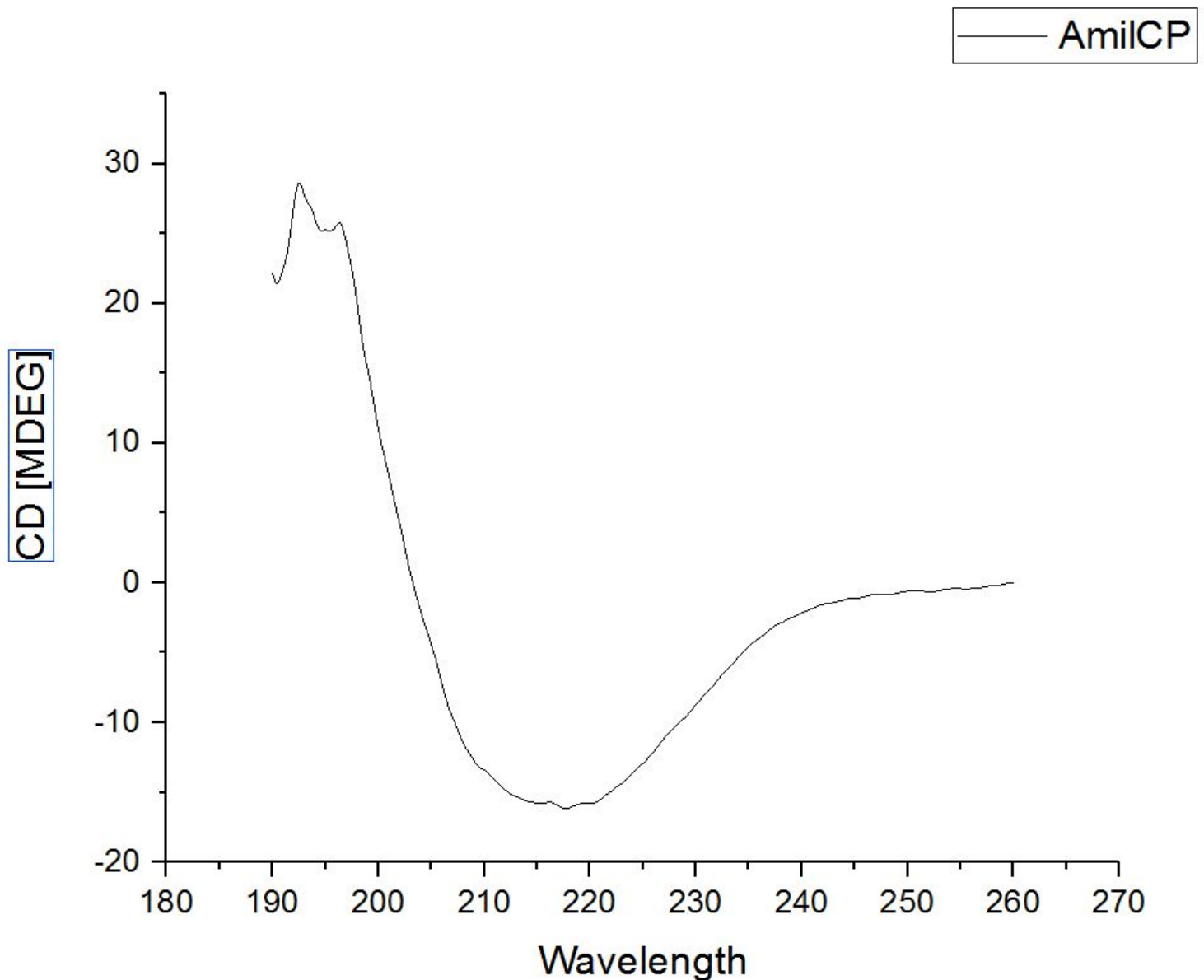


Figure 1. Circular Dichroism Spectrum of AmilCP in 50mM Phosphate Buffer

Analysis of the interaction of circularly polarized light with AmilCP indicates that the secondary structure is predominantly composed of β -sheets. The minima broadening towards 210nm suggests that there are discrete α -helix

structures in the proteins but are not a significant feature of this protein. The effect of the β -sheets prevents any smaller features from being characterized. Furthermore, a negative of polarized light followed by a positive rotation of polarized light suggests that the β -sheets are arranged in an anti-parallel fashion throughout the protein.

References

1. Allen, James P. *Biophysical Chemistry*. 1st ed. Wiley-Blackwell. 2008.
2. Van Holde, K.E; et. al. *Principles of Physical Biochemistry*. 2nd ed. Pearson. Upper Saddle River, NJ. 2006