Circular Dichroism of Protein-Dye Complexes as a Characterization Tool for Protein Higher Order Structures

Christopher Sucato, Shreya Lahate, Libo Wang, and Mario DiPaola

Blue Stream Laboratories, 8 Henshaw Street, Woburn, MA 01801  csucato@bluestreamlabs.com  617-234-0001

ABSTRACT

The near-UV dichroism of BSA and ANS complexes, as a Function of BSA Purification Method

CD spectroscopy plays an important role in the determination of protein structures. As a means to examine purified proteins for structural integrity and proper folding, and to study relevant conformational changes in response to changes in pH, temperature, and other modifying variables. During production of biological samples, CD spectroscopy can provide a non-invasive and non-destructive means of monitoring stability and checking batch-to-batch consistency of the drug product. CD spectroscopy in the near-UV wavelength region provides a unique fingerprint of aromatic residues and doublet bridges. Although the data from near-UV CD cannot be quantitated in the same manner as far-UV CD, it can be used to study conformational changes in proteins. The fingerprint obtained from near-UV CD is useful as a characteristic marker of protein tertiary structure, particularly in the region of aromatic residues.

BACKGROUND:

Here we present the results of circular dichroism studies of protein-ANS complexes that red-shift and produce a positive peak at 290 nm. We also report CD studies of BSA of 1 mg/mL, with a stock solution of 3 mg/mL prepared from this stock. Buffer used for dilutions and CD preparation was PBS pH 7.4. The samples prepared above were then incubated at 25 °C for one hour before being tested. The CD spectra were collected on a JASCO 715 spectropolarimeter with parameters as follows: wavelength range: 250-450 nm; data pitch: 0.5 nm; scan speed: 20 nm/min; response: 8 scans; bandwidth: 1.0 nm.

RESULTS AND DISCUSSION

To further investigate the enhancement of CD signal upon ANS binding, we studied the near-UV CD data as a function of the BSA secondary structure, and the effect of ANS binding on the secondary structure of BSA. We found that the enhancement of CD signal is due to a close association of ANS with aromatic residues that contribute to CD in the region of 250-280 nm. This is a clear indication of the enhancement of CD signal due to the formation of a BSA/ANS complex that has a dynamic non-covalent association in the thermodynamic equilibrium, but at the same time, it is an interesting effect that can be observed.

The samples were prepared in similar fashion as the initial sample. The samples were prepared 4% mg/mL, as it gave good results in the previous study. Two sets of samples were prepared. Both sets were first incubated at 25 °C for one hour before being tested. One set was then refrigerated at 4 °C and the other set was tested immediately.

CONCLUSIONS

As shown by the temperature study, a small but significant temperature effect is observed, consistent with an equilibrium binding effect of ANS in hydrophobic pockets stored by aromatic residues that have a dipole. A study that we report is related to the point mentioned above, that the significant ANS-induced near-UV signal may have a practical use as a sensitive probe for structural differences between protein samples. In the section below is shown the results of a comparison of near-UV CD data of two different sets of BSA. The sets-BSA studies are both commercially available BSA, both tested at greater than 90% purity, but were purified by different methods. We conducted protein binding studies in the presence of protein binding affinities using a spectrophotometric method (BIC assay). The results are shown in Figure 6 above, and reveal that only slight differences in the spectra of BSA with ANS bound to the single Tyr on the native protein and also after incubation with ANS dye.

REFERENCES


ACKNOWLEDGMENTS

We would like to acknowledge all of the members of blue stream laboratories in support.

The work presented here is intended to be used for diagnostic purposes in animals or veterinary field. The therapeutic implications have not been the subject of any investigations or clinical trials whatsoever.

Figure 1: The chemical structure of ANS, and the three aromatic amino acids tyrosine, tryptophan and phenylalanine. Four amino acids, tyrosine, tryptophan and phenylalanine, are red for aromatic residues, blue for the carbohydrate moiety, and yellow for the aromatic ring.

Figure 2: Measurement of a CD spectra as illustrated in the upper panel. A beam of monochromatic light is passed through a test cell. A polarized light is passed through the sample and due to the absorption is different between left and right light. The differential absorbance results in the CD spectra. Lower panel: Infrared or visible (red) only regions of a protein will not give rise to CD signal because of a lack of chirality on this scale size of the molecule. Folded domains however, are likely to be asymmetric, and thus, will contribute to right- and left-handedness on a CD scale.

Figure 3: Ribbon structure of BSA, the yellow plane slicing structures are ribbons where fatty acids are located and red and blue are structurally important. The CD primary sequence is shown on the right, when the aromatic CD active residues are highlighted. (Image courtesy of D. Kroschwitz et al., 2001).

Figure 4: Upper Panel: near-UV CD data for BSA alone and BSA bound with ANS dye. CD signal in the region of tryptophan and phenylalanine with ANS dye is evident. In the inset is shown the sensitivity of ANS dye to the signal.

Figure 5: BSA and BSA-ANS near-UV CD spectra as a function of protein concentration. The combination of the CD signal is in the region 260-270 nm correlates with the temperature in a manner consistent with a reversible association of ANS in proximity to the Tyr residues.

Figure 6: CD spectra of ANS dyes. The BSA samples with ANS dyes show similar CD peaks with slight differences in ANS binding.

Near-UV CD Spectra of BSA and BSA-ANS complexes, as a Function of BSA Purification Method

Near-UV CD Spectra of BSA and BSA-ANS complexes, as a Function of BSA Purification Method

Near-UV CD Spectra of BSA and BSA-ANS complexes, as a Function of BSA Purification Method