Expt. No 1 Date

Page No. 1

Experiment - 1

Aim: To calculate the number of tyrosine and tryptophan residues in the given BSA samples using UV - Vis spectrophotometer.

Theory: UV-Vis spectroscopy is an analytical technique that measures the amount of discrete wavelengths of UV or visible light are absorbed by or transmitted through a sample in comparison to a reference or blank sample. This property is influenced by the sample composition providing information on the concentration of the sample

Light has a certain amount of energy which is inversely proportional to its wavelength. A specific amount of energy is needed to promote electrons in a substance to a higher energy state which we can detect as absorption. This is why absorption of light occurs for different wavelengths in different substances

In this experiment, BSA sample is denatured using 8M urea and its absorbance is measured at 280nm and 288 nm. From the information, the value of extinction coefficient is calculated for both 280 nm and 288 nm absorbance values along with the number of tyrosine and tryptophan residues. From the given BSA sequences, the number of tyrosine and tryptophan residue per protein are calculated

Teacher's Signature

Date

Expt. No.

Page No. 2

Material Required: BSA sample, 8M urea, 1X PBS (prepared from 10x PBS stock solution by adding 22.5 ml water to 2.5 ml 10X PBS), cuvette, spectrophotometer.

Procedure:

1. At first, 1X PBS is made from 10X PBS stock solution.
2. In spectrophotometer instrument blank is set up with 1XPBS in cuvette to be zero at 280 nm.
3. BSA is now dissolved in PBS.
4. Absorbance is measured at 280nm with native BSA dissolved in PBS.
5. Now 8M urea (12.016 g of urea in 25 ml 1XPBS) solution is made.
6. Now blank is set to zero with 8M urea solution (urea + 1X PBS)
7. Then BSA is denatured by dissolving in 8M urea solution.
8. Absorbance of the denatured BSA solution is measured at 280 nm
9. The above procedure is repeated and OD is taken at 288 nm as well.

Observation Table:

Wavelength	PBS (Blank)	PBS + Native BSA	Blank - PBS + Urea	Denatured BSA (+ urea)
A (280 nm)	0	1.011	0	0.699
A (288nm)	0	0.708	0	0.370

Teacher's Signature

Expt. No. _____ Date _____

Page No. 3

Calculations: $c = (1g/l) / 66463 = 1.505 \times 10^{-5}$

$l = 1cm = 10^{-2} m$

For 280nm, $A_1 = \varepsilon_1 cl$

$$\varepsilon_1 = \frac{A_1}{cl} = \frac{0.699}{1.505 \times 10^{-5} M \times 1cm}$$

$$\varepsilon_1 = 4.646 \times 10^4 \ M^{-1}cm^{-1}$$

For 288nm,

$$A_2 = \varepsilon_2 cl$$

$$\varepsilon_2 = \frac{A_2}{cl} = \frac{0.370}{1.505 \times 10^{-5} \times 1cm}$$

$$\varepsilon_2 = 2.460 \times 10^4 \ M^{-1}cm^{-1}$$

ε_1 (280nm) $= N_{Trp} \times 5690 + N_{Tyr} \times 1280$

$4.646 \times 10^4 = N_{Trp} \times 5690 + N_{Tyr} \times 1280$ —— (1)

ε_2 (288nm) $= N_{Trp} \times 4815 + N_{Tyr} \times 385$

$2.46 \times 10^4 = N_{Trp} \times 4815 + N_{Tyr} \times 385$ —— (2)

(1) $\times$ 4815 − (2) $\times$ 5690

$$N_{Tyr} = \frac{8376.893994 \times 10^4}{3972550} \approx 21$$

Teacher's Signature _____

Expt. No. _____

Page No. 4.

$N_{Tyr} \approx 21$

Putting N_{Tyr} in eq^n (1)

$$N_{Trp} = \frac{19577.622872}{5600}$$

$N_{Trp} \approx 3$

Conclusion:

The values of extinction coefficient are $4.646 \times 10^4 \, M^{-1}cm^{-1}$ and $2.46 \times 10^4 \, M^{-1} cm^{-1}$ at 280 nm and 288 nm respectively.

The number of tyrosine and tryptophan residues is equal to 21 and 3. Absorbance by protein in denaturated form is less than native.

Discussion:

In UV - Vis spectroscopy the wavelength corresponding to the maximum absorbance of the sample is chosen. This ensures maximum sensitivity because the largest response is obtained for a certain analyte concentration. The technique allows samples to be reused. The instrument is easy to use in which measurements can be made easily.

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