EXPERIMENT II: PROTEIN LIGAND INTERACTIONS: BINDING of COUMARIN COMPOUNDS to PROTEIN

Theory: If there is a nonpolar molecular group on one surface (phenyl ring, hydrophobic alkyl chain, etc.), the molecular group in the contact area on the other surface should also be hydrophobic and nonpolar. If there is a charge on one surface (+), the neutralizing charge must be on the other surface. In short, two molecules must be chemically competing.

Spectral measurement is taken to measure the dynamics of ligand macromolecular interactions, that is, a simple UV and visible field spectrophotometer is sufficient. Whether absorption or fluorescence can be detected if binding can produce a spectral change.

When the 4-OH coumarin compound binds to protein, the compound undergoes a spectral change that is quite apparent in the visible area. The spectrum of the free compound is around 340 nm. When the compound and protein are mixed, a new absorption band appears. The increase in absorption at 340 nm is directly related to the interaction with protein. The new absorption band belongs to the coumarin - protein complex. At 280 nm, the protein band, change occurs after interaction with the coumarin compound.

Solutions and Chemicals Used in the Experiment:

- Solution A: Phosphate buffer 0.01 M pH: 7.4
- Solution B: 10 ml methanol + 90 ml A solution
- Solution C: 0.001 g coumarin + 50 ml B solution
- Solution D: 0.05 g BSA 10 ml A solution

➢ $Mw_{coumarin}$: 162.14 g/mol, Mw_{BSA}: 66430.3 g/mol

1. 500 µL Solution D + 2500 µL Solution A
2. 500 µL Solution D + 200 µL Solution C + 2300 µL Solution A
3. 500 µL Solution D + 400 µL Solution C + 2100 µL Solution A
4. 500 µL Solution D + 600 µL Solution C + 1900 µL Solution A
5. 500 µL Solution D + 800 µL Solution C + 1700 µL Solution A
6. 500 µL Solution D + 1000 µL Solution C + 1500 µL Solution A
7. 500 µL Solution D + 1200 µL Solution C + 1300 µL Solution A
8. 500 µL Solution D + 1400 µL Solution C + 1100 µL Solution A
9. 500 µL Solution D + 1600 µL Solution C + 900 µL Solution A
10. 500 µL Solution D + 1800 µL Solution C + 700 µL Solution A
11. 500 µL Solution D + 2000 µL Solution C + 500 µL Solution A
12. 2400 µL Solution A + 600 µL Solution C

Before measure spectrum the spectrophotometer (double beam) was reset with solution A. All spectrums of solutions between 180-600 nm are taken.

$A_0/(A-A_0)$ (y-axis) versus 1/c (x-axis) is drawn (Don't forget to examine the R^2 value of the graph. It gives information about the accuracy of the graph). The slope of this graph gives Ka. ΔG was calculated using the Gibbs-Helmholtz equation (below).

$$\Delta G = -RT\ln Ka \quad R = \text{Universal gas constant- } 8.314\ JK^{-1}mol^{-1},\ T = 298\ K$$

Interpret the results based on these data.