Using Spectrophotometry to analyse protein structures and its amino acid residues

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January 16, 2018

Abstract

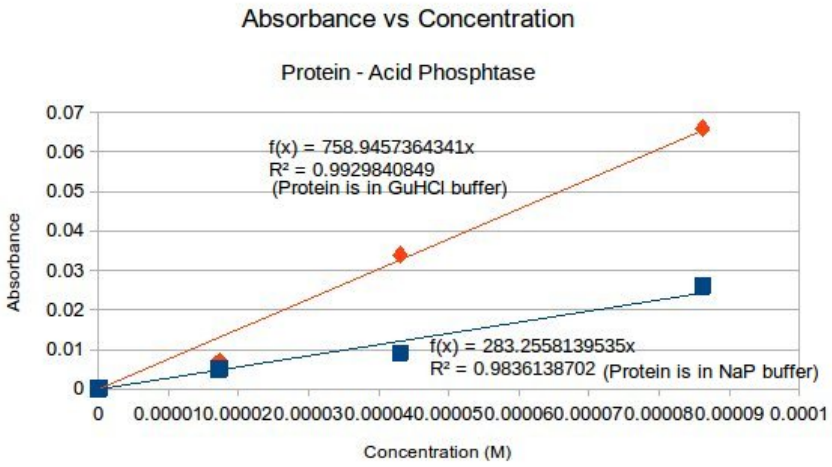
Proteins in general are found to show an absorption maxima at the wavelength range of 275nm - 280 nm. The side chains of amino acid residues, which play significant roles in absorbance of light are that of Tryptophan and Tyrosine. In addition to that, evident from spectrophotometric analysis, disulfide bonds formed by cysteine residues, in a protein, are also found to show similar absorption spectra.

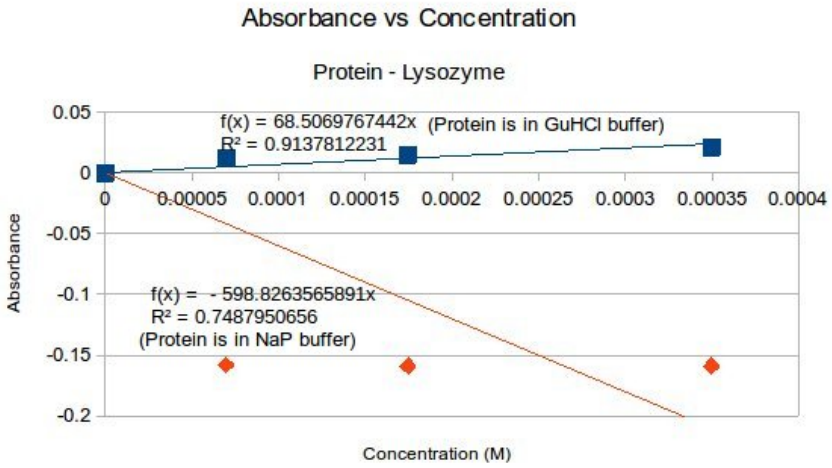
The purpose of this study is to experimentally determine the λ_{max}(wavelength corresponding to the maximum absorption) and find molar extinction coefficient of native and unfolded proteins. In addition to that, this study also aims at determining the concentration of free sulfhydryls(thiols groups) in the native and unfolded protein. In buffer 1(Sodium Phosphate in EDTA) proteins remain in their folded, native form but in buffer 2(GuHCl) proteins unfold.

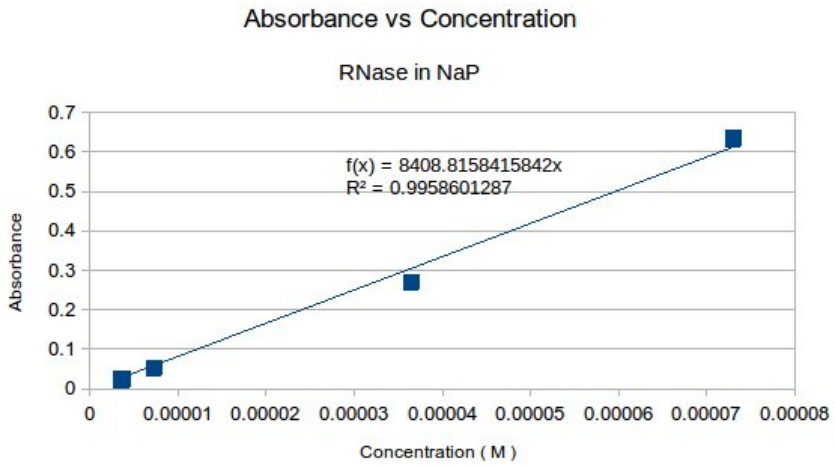
Absorbance is basically the measure of the attenuation of light intensity after an incident light crosses a barrier (a solution, in this case). As per the Beer-Lambert's law, Absorbance has a linear relationship with the Concentration of a solution, through which light is passing. Tyrosine and Tryptophan residues in the protein are expected to attenuate the intensity of the incident light, by absorbing the energy from the incident photons. A spectrophotometer is used to determine the value of the λ_{max} and the corresponding absorbance value. The same procedure is performed for different concentrations of the protein in folded form (in NaP buffer) and unfolded form (in GuHCl buffer).

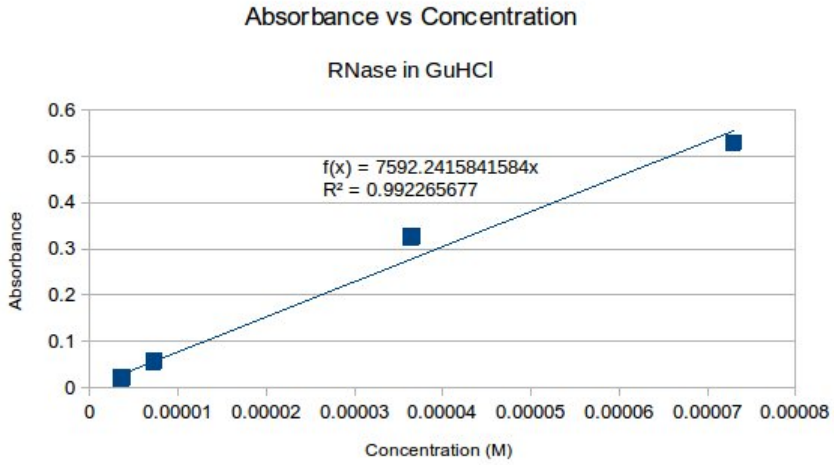
The experiment deals with the Ellman's Reaction, which would help us in reaching our goal. Ellman's reagent forms disulfide bond with the free thiol groups present in the solution, liberating a chromophore, 5-mercapto-2-nitrobenzoic acid, whose absorption maxima is at 412 nm. The incubated mixtures of Ellman's reagent and the protein solutions (of different concentrations) were put in a spectrophotometer with a light of wavelength 412 nm and the values of absorbance for the chromophore were measured. This procedure is performed for both folded and unfolded forms, for both proteins.

Results:


Absorbance vs Concentration
Protein - Acid Phosphtase
f(x) = 758.9457364341x
R² = 0.9929840849
(Protein is in GuHCl buffer)
f(x) = 283.2558139535x
R² = 0.9836138702
(Protein is in NaP buffer)
Absorbance
Concentration (M)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0 0.00001 0.00002 0.00003 0.00004 0.00005 0.00006 0.00007 0.00008 0.00009 0.0001


Absorbance vs Concentration
Protein - Lysozyme
f(x) = 68.5069767442x
R² = 0.9137812231
(Protein is in GuHCl buffer)
f(x) = - 598.8263565891x
R² = 0.7487950656
(Protein is in NaP buffer)
Absorbance
Concentration (M)
0.05
0
-0.05
-0.1
-0.15
-0.2
0 0.00005 0.0001 0.00015 0.0002 0.00025 0.0003 0.00035 0.0004





Ans. The value of λ_{max} is 277nm for the protein in sodium phosphate-EDTA buffer whereas, the value is 276nm for the protein in guanidine hydrocholride buffer. These values are indicative of the fact that the solution bears aromatic rings and hence, most likely, the protein has tyrosine and tryptophan residues.

Yes, the value of λ_{max} did change in the presence of guanidine chloride but just by 1 nm.

2. Is there a difference in the experimental molar extinction coefficients in neutral buffer and in 6M guanidine hydrochloride? Why or why not?

Ans. Yes, there was indeed a difference in the experimental molar extinction coefficients in neutral buffer and in 6M GuHCl.

The possible explanation to support this observation would be by the difference in the concentration of chromophores released, after the Ellman's reaction had occurred, in the two different buffer solutions. While unfolded, the protein is expected to perform the reaction more, whereas, the folded protein won't react much as it is expected to form disulfide bonds with residues within itself.