EXPLORING THE POTENTIAL EFFECTS OF CHOLESTEROL: A SPECTROSCOPIC STUDY

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ABSTRACT

Detection of cholesterol is very important for diabetes management. Though it is the energy source in our body, the increasing cholesterol level leads to chronic metabolic disorder finally ends in blindness, nerve degeneration and kidney failure. Although cholesterol is an essential component for mammals, higher levels of cholesterol in blood have been linked to damages to arteries and diseases associated with the cardiovascular system. Therefore always there is an increasing demand in the development of new sensor elements / sensor design with more sensitivity and durability for the detection of cholesterol.

1. INTRODUCTION

Generally non-enzymatic electrochemical detection method is the most preferred method. [1] Electrospun naofibers, carbon nanotubes and bimetallic nano composites are also widely used for cholesterol detection but lacks stability of the biocomponent. [12]Here we report the possibility of cholesterol detection using proteins like BSA(Bovine serum albumin), Human serum albumin(HSA), ovalbumin and lysozyme immobilized polyelectrolyte multilayer membranes fabricated on a polymer support through layer by layer assembly. Faster sensing of chemical and biochemical agents remains an ever expanding area with the demand of resolution at attogram level. The sensing efficiency depends on the effective interaction between the sensor and the analyte. [3] In order to achieve high efficiency there is a general trend to develop high surface area sensor interface.

LbL is such a versatile approach that impart high surface area to volume ratio to membranes along with the ability to locate the fluorophore to the surface. [4] Self assembled multilayers often provide a safe electrostatic cage for the embedded biomolecules where in their bioactivity is preserved. Thus providing high surface area to volume ratio and strong interaction with analyte molecules. The interaction of cholesterol with these proteins was investigated using Steady state fluorescence spectroscopy, Ultra-violet visible absorption, Synchronous fluorescence, Time resolved fluorescence and FTIR measurements. Protein immobilized nanolayers are seemed to respond very fastly to the micro molar concentration of the analyte. [5]

2. MATERIALS AND METHODS

1.Fabrication of polyelectrolyte nanolayers

The substrate supporting membrane (nylon, supor) was rinsed with Millipore water and kept in water for 24 hours. The clean membrane was dipped in alternate polycationic and polyanionic solutions for 15 minutes. After each dipping, the membrane was rinsed with 50 ml distilled water for 1 minute. The above steps were repeated till required number of bilayers were formed (6 - 9 bilayers). A similar way is adopted for the fabrication of protein – polyelectrolyte nanolayer formation. ovalbumin, solution (0.25 mg/ml,) prepared using different buffer pH [(3.0, 4.5, 8.8 & 10.6) were prepared using citric acid – sodium citrate, tris-HCl and Glycine-NaOH buffer] can be immobilized to polyelectrolyte nanolayers through ultra filtration (UF) at room temperature (28- 30^0C) and through simple adsorption.

2.Film characterization

The self assembled CHI/PSS nanolayers can be characterized using FTIR (Fourier Transform Infra-red) Spectroscopy, from the sulfonate peak height (SO_3^-), at 1033 cm^-. Protein deposition onto polyelectrolyte nanolayers can be analyzed using FITR (from amide I band, 1650 cm^{-1}), UV/VIS absorption studies can be carried out at a wavelength range of 200-900 nm.

3. Fluorescence measurements

Steady state and synchronous fluorescence measurements can be carried out using a spectofluorometer at an excitation wavelength 295 nm with emission spectral data in the range 300-500 nm. Fluorescence excitation spectrum can be recorded in the wavelength range 200-350 nm at an emission wavelength 340

nm. Stock solutions of analyte molecules (cholesterol) can be prepared at a concentration of 1×10^{-1} M and desired concentrations will be achieved by successive dilution. The synchronous fluorescence measurements can be carried out at an excitation wavelength of 290 nm keeping a constant wavelength difference of $\lambda = 15$ nm and $\lambda = 60$ nm for tyrosine and tryptophan residues respectively.

3. RESULTS AND DISCUSSION

UV/ VIS Absorption Studies

To study the effect of cholesterol on ovalbumin molecule UV/ VIS absorption studies are carried out by varying cholesterol concentration. Since, UV/VIS spectroscopy is a reliable method to explore the structural changes under gone by protein molecule and to understand about the complex formation . Ovalbumin possess an absorption maximum at 280 nm corresponding to TRP 148, 184 and 267. Cholesterol is known to quench tryptophan fluorescence by collisional process.[6]

Theconcentration of analyte molecules was varied from micro molar to atto molar level.Fig. 1, displays the UV/VIS absorbance spectra of ovalbumin at different contents of cholesterol.

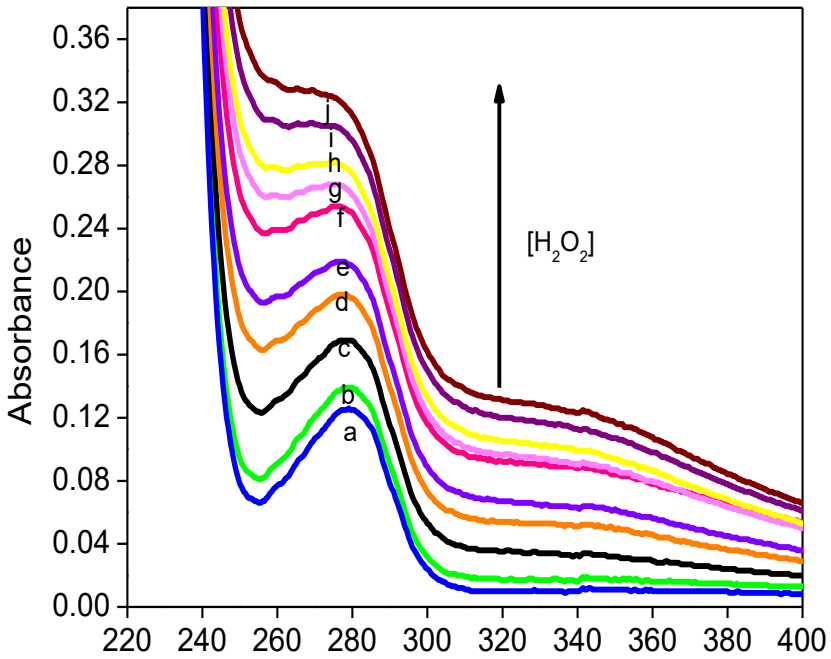

Fig 1 UV-VIS absorption spectrum of ovalbumin solution - hydrogen peroxide system. at pH 4.5. a: [ovalbumin] = 5.5 x 10^{-6} mol L^{-1} , b-j: hydrogen peroxide concentration in the range 10^{-6} mol L^{-1}.

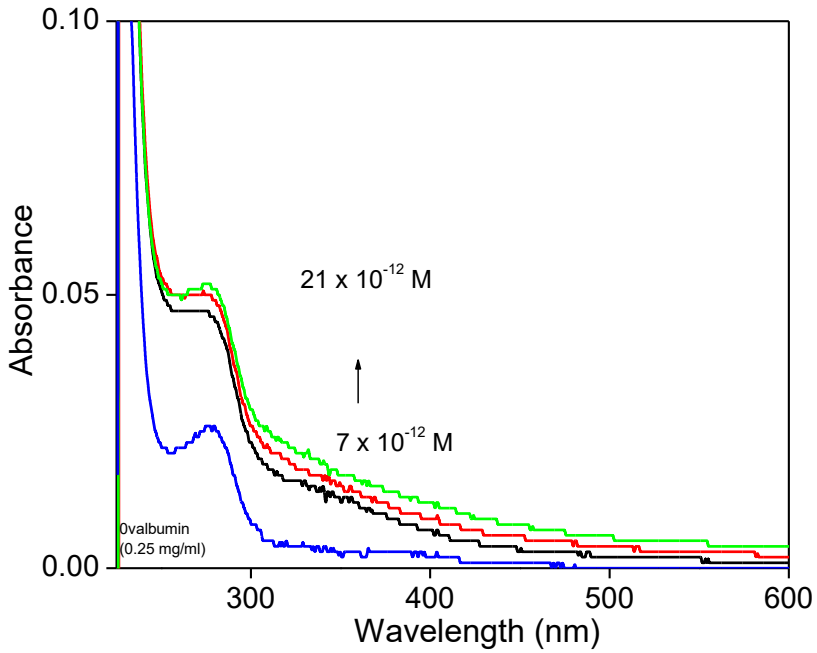

Figure:1 UV-VIS absorption spectrum of ovalbumin solution - cholesterol system. at pH 4.8. a: [ovalbumin] =(0.25 mg/ml), : [Q]= 7 x $10^{-12}$to 21x 10^{-12}M.

It is clear from the UV/VIS absorbance spectra that the peak at 280 nm was raised and the absorption spectral maximum shows a slight blue shift (from 280 to 276 nm). Which means the protein microenvironment was changed on interaction with cholesterol, and resulted in the formation of cholesterol –ovalbumin complex. Ovalbumin exhibits a concentration-dependent enhancement in absorption intensity on varying quencher concentration, due to the exposure of the chromophoric amino acid residues in ovalbumin to a highly polar environment (cholesterol),[7] it again mean that the hydrophobicity around protein molecule was decreased.Since there is a change in the absorption spectra of ovalbumin in presences of cholesterol, we have to follow the changes in the intrinsic fluorescence of ovalbumin in presence of cholesterol.

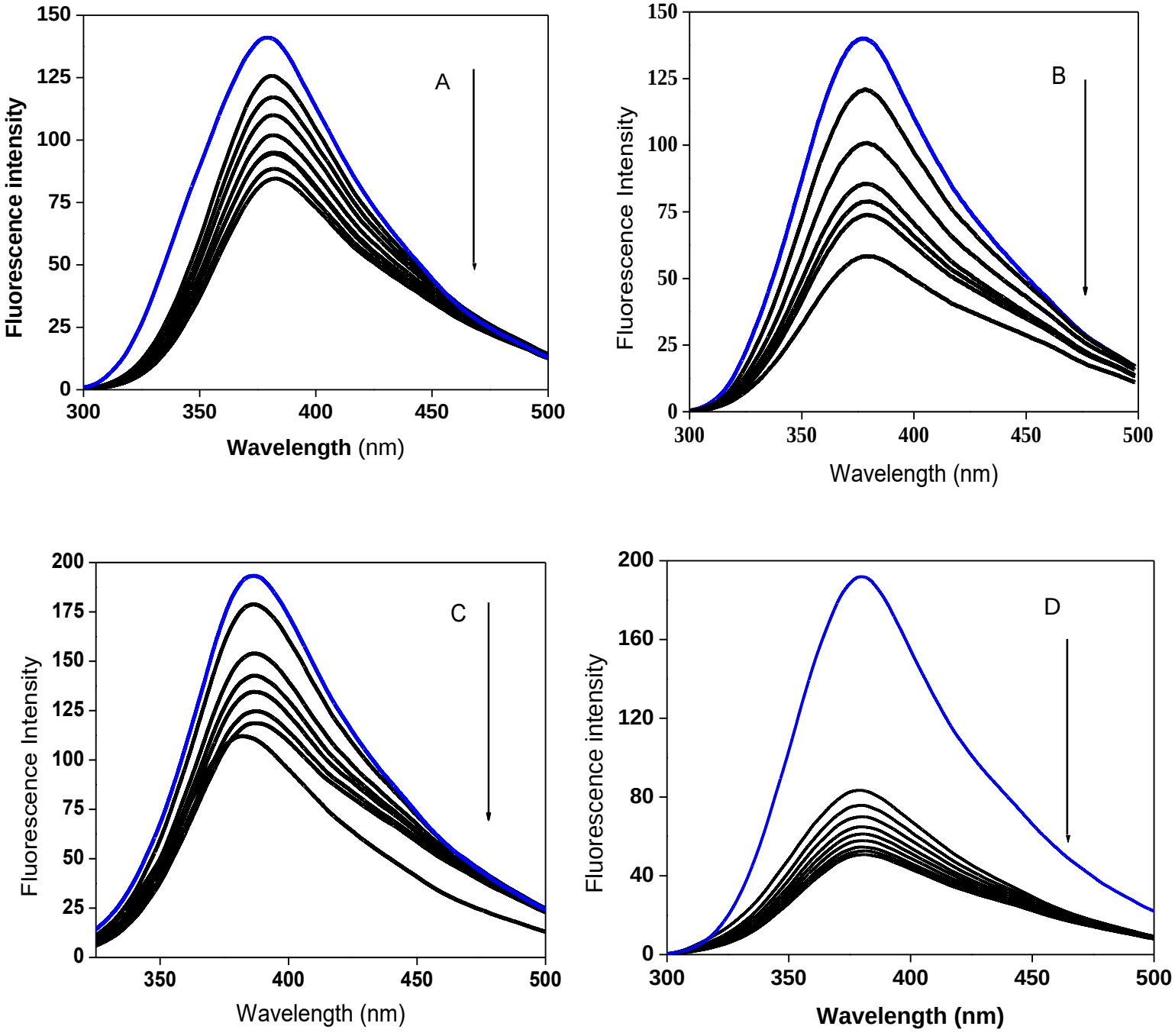

Figure:2 Fluorescence emission spectrum of OVA-CHI / PSS nanolayer in presence of cholesterol (10^{-6}mol L^{-1}) . A: pH 3.0, B: pH 4.5, C: pH 8.8, D: pH 10.6

Steady state fluorescence measurements regarding protein structure and binding interactions (such as binding modes, binding constants, binding sites, quenching rate constants, etc) are carried out by varying cholesterol and glucose concentration.

4.CONCLUSION

These studies point out that CHI/PSS/SUPOR woks as a sensor for cholesterol selectively and variations undergone by the protein microenvironment can be clearly monitored upto 10^{-12} mol L^{-1}. These observations and studies clearly shows that that it is possible to create a biosensor for the determination of cholesterol using protein immobilized nanolayers. The lower limit of detection for our system is $[H_2O_2]= 10^{-12}$ mol L^{-1}. Moreover only few bilayer depositions on porous polymer support is sufficient to sense cholesterol up to this particular concentration.

KEY WORDS

OVA- Ovalbumin

SUPOR- Polystyrenesulfonate membrane

CHI-Chitosan

PSS-Polystyrenesulfonate

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REFERENCE

1	Decher., G, Hong., J.D, Schmitt., J , Thin Solid Films, 1992 (210) 831.
2	Xianyan, W., Christopher., D, Lee., H, K, Senecal .,J, Kumar .,J. Samuelson .,L. A , Nano Lett. 2002(2)1273.
3	Wang.,X, Kim.,Y, Drew.,C, Ku.,B.C, Kumar., J, Samuelson., L. A, Nano Lett. 2004 (4) 331.
4	Lee,H., Kumar., J , Tripathy., S.K., Langmuir. 2000, (16), 10482.
5	Li.,X, Feyter., S. D.E, Chan.,D, Alda.,S, , Filip.,P.V, Prez., D.U ,. Vankelecom., F.J, , Chem Mater. 2008 (20) 3876.
6	Aravind., U.K, Mathew., J, Aravindkumar., C.T, J.Membrane science 2007 (299) 146.
6	Lackowicz., J.R, Plenum Press: New York, **1983**.
7	Vijayalakshmi.,A, Karthikeyan .,R, Berchmans., S, J.Phys.Chem.C, **2010**, (114) 22159.
8	Kurzawa., C, Hengstenberg .,A, Schuhmann .,W, Anal. Chem, **2002**, (74), 355 .
9	Nagaraja., P, Shivakuma .,A, Shrestha., A. K, Anal. Biochem, **2009**, (395), 231.
10	Li .,L, Du., Z, Liu., S, Hao.,Q, Wang .,Y, Li .,Q, Ng., T. W, Talanta **2010,**(82), 1637.