Biosensors and Bioelectronics 25 (2010) 1149–1154

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Synchronous fluorescence based biosensor for albumin determination by cooperative binding of fluorescence probe in a supra-biomolecular host–protein assembly

Digambara Patra*

Department of Chemistry, Faculty of Arts and Sciences, American University of Beirut, P.O. Box 11-0236, Riad El Solh, Beirut, 1107-2020, Lebanon

ARTICLE INFO

Article history:
Received 15 July 2009
Received in revised form
14 September 2009
Accepted 30 September 2009
Available online 9 October 2009

Keywords:
Albumins
Host–guest assembly
Fluorescence probe
Biosensor
SFS

ABSTRACT

A synchronous fluorescence probe based biosensor for estimation of albumin with high sensitivity and selectivity was developed. Unlike conventional fluorescence emission or excitation spectral measurements, synchronous fluorescence measurement offered exclusively a new synchronous fluorescence peak in the shorter wavelength range upon binding of chrysene with protein making it an easy identification tool for albumin determination. The cooperative binding of a fluorescence probe, chrysene, in a supramolecular host–protein assembly during various albumin assessments was investigated. The presence of supramolecular host molecules such as β-cyclodextrin, curucurbit[6]uril or curucurbit[7]uril have little influence on sensitivity or limit of detection during albumin determination but reduced dramatically interference from various coexisting metal ion quenchers/enhancers. Using the present method the limit of detection for BSA and γ-Globulin was found to be 0.005 μM which is more sensitive than reported values.

1. Introduction

Albumin comprises about half of the blood serum protein. It is soluble and monomeric. Patient with kidney disease, diabetes, or hypertension and metabolic syndrome should be screened for albuminuria (Ruhn et al., 1994). There are a variety of methods for assessing urinary albumin excretion, extending from the very low-range microalbuminuria to higher ranges extending into macroalbuminuria or proteinuria (Babic et al., 2006; Baczynskyj et al., 1994; Busby and Bakris, 2004; Chan and Herold, 2006; Comper and Osicka, 2005; Hirayama et al., 1990; Kleinpeter, 2007; Singh et al., 2007; Toto, 2004; Walcher et al., 2003; Zhang et al., 2003). Fluorescence methods are known to be highly sensitive and selective. Synchronous fluorescence spectroscopy (SFS) (Patra and Ghaddar, 2009; Patra and Mishra, 2002a), a method where both excitation and emission monochromators are scanned simultaneously while keeping a fixed wavelength interval between them, constant $\Delta\lambda$ mode (Patra and Mishra, 2002a) is highly selective compared to conventional fluorescence emission or excitation spectral measurement. In SFS, appreciable results are obtained mainly due to narrowing of spectral band, simplification of emission spectra, and contraction of spectral range. From an analytical view point, the whole spectrum might not be of much interest, and many of the details may not generally be considered; their presence results only in confusing the total spectrum because of the overlap with the emission of other components in the sample. So a narrower and simplified spectrum in contraction spectral range can provide high selectivity for identification and estimation compared to a broad emission or excitation spectrum. SFS has been successfully used for the simultaneous determination of various systems (Patra and Mishra, 2000a,b, 2001, 2002b).

Fluorescent properties of probe molecules through host–guest assemblies or associations with protein enable biomedical sensor applications and improve the factors governing molecular recognition. Macrocyclic hosts impose a structural confinement of the included chromophoric guest molecules, increase their thermal and photochemical stability, and isolate them from the bulk water, which together improves their radiative properties. Owing to the importance of fluorescent probes and sensors for environmental and biological applications, it is usually desirable to "tune" fluorescent dyes in aqueous solution, which places the emphasis on the investigation of water-soluble hosts. The most common macrocyclic hosts like cyclodextrins (Bortulus and Monti, 1996; Zhang et al., 2002) and calix[*n*]arenes (Abraham, 2002; Diamond and McKervey, 1996; Ikeda and Shinkai, 1997) have been studied in this context. Cucurbit[*n*]uril, a synthetic pumpkin-shaped cation receptor, has recently attracted much attention because of its remarkably strong binding of organic dyes, protonated alkyl and aryl amines,

* Tel.: +961 1350 000x3985; fax: +961 1365217.
E-mail address: dp03@aub.edu.lb.

0956-5663/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2009.09.041

and metal ions. CB6 and CB7, in particular, are known for its exceptional thermal and photochemical stabilization of fluorescent dyes. Fluorescence enhancement upon encapsulation into cucurbiturils in solution was first observed and documented by Wagner and coworkers (Rankin and Wagner, 2004; Wagner et al., 2001, 2003). The authors observed an enhancement of 5 times for both curcumin and 2-anilinonaphthalene-6-sulfonate (2,6-ANS) with CB6 and ca. 25 times for 2,6-ANS with CB7. The enhancement for 1-anilinonaphthalene-8-sulfonate (1,8-ANS) with CB7 was larger. Nau group has extensively work on fluorescence enhancement, life time prolongation, and photostability of guest molecule in cucurbit[7]uril and study the polarity and polarizability of cucurbit[7]uril cavity (Bhasikuttan et al., 2007; Hennig et al., 2007; Koner and Nau, 2007; Marquez et al., 2004; Maĭrquez and Nau, 2001; Mohanty et al., 2006, 2008; Mohanty and Nau, 2004, 2005; Nau and Mohanty, 2005; Shaikh et al., 2008).

Polycyclic Aromatic Hydrocarbons (PAHs) molecules such as chrysene are known to be fluorescent due to their rigid structure. They have very high fluorescence quantum yield, longer fluorescence lifetime and are hydrophobic in nature making them versatile for optical and biomedical sensor development to assay biological systems and proteins (Patra and Mishra, 2006). PAHs like chrysene has extra advantage to overcome short wavelength fluorescence from impurity present in biological sample that could interfere in fluorescence measurement. Fluorescent probe, chrysene, along with cooperative binding of cucurbituril could act as a potential enhancer towards biosensor development for efficient monitoring of albumins as the binding affinity to a biomacromolecule, such as albumin can be synergetically enhanced by addition of a host molecule like cyclodextrin or cucurbituril. This opens up new opportunities to improve the biological and therapeutic activity of organic dyes as well as for biosensor development by the addition of macrocyclic hosts as supramolecular "enhancers" or "protector". In this paper, SFS technique is applied for albumin determination by cooperative binding of chrysene in a supra-biomolecular host–protein assembly. The sensitivity found for such estimation is higher than reported values and supramolecular host molecule acts as protector from coexisting foreign metal ions during biosensing of albumins.

2. Material and methods

2.1. Sample

Bovine Serum Albumin (BSA), Globulin (γ-G), Cucrurbit[6]uril (CB6) and β-Cyclodextrin (β-CD) were obtained from Fluka, where as Human Serum Albumin (HSA) and Cucurbit[7]uril (CB7) were from Sigma and Aldrich respectively. Sock solution of chrysene (Acros) was prepared in acetronitrile (Acros) and then diluted in buffer solution. The buffer solution was made of phosphate buffer at pH 7.0 and was prepared by taking different concentration of K_2HPO_4 and KH_2PO_4 using standard laboratory method. The final concentration of phosphate in the buffer solution was 50 mM. Stock solution of BSA, HSA, γ-G and β-CD was prepared in phosphate buffer, whereas in case of CB6 and CB7 stock solution was made in 0.2 M Na_2SO_4 and the solution was further diluted in phosphate buffer pH 7.0. Throughout the measurement pH of the buffer was maintained at 7.0. Various concentrations of protein samples were prepared by taking desired volume from the stock sample and diluting it in buffer solution in the presence of chrysene with or without host molecule such as β-CD, CB6 or CB7. It was made sure that amount of acetronitrile present in the sample is always constant and less than 1% (v/v). The spectroscopic measurements were performed on the fresh samples at room temperature. The photostability of the chrysene was reasonable during the entire measurements. The experiment on interference of coexisting foreign substances was carried out by taking albumin and chrysene in the absence and presence of various metal ions in micromolar concentration range. The SFS intensity of chrysene along with albumin, with and without metal ion, was measured and ratio of SFS intensity in the absence and presence of metal ion was calculated. Similarly, the experiment was repeated in the presence of host molecule along with albumin and chrysene, with and without metal ion, and the ratio of SFS intensity was calculated. Both the results with and without host molecule were compared.

2.2. Instrumentation

Absorbance measurements were done using a JASCO V-570 UV/vis/NIR spectrophotometer. Fluorescence measurements were obtained on a JOBIN YVON Horiba Fluorolog 3 spectrofluorometer. The excitation and emission slits width were 5 nm. The excitation source was a 100 W Xenon lamp. The detector used was R-928 operating at a voltage of 950 V. The synchronous fluorescence spectra were measured at $\Delta\lambda$ = 5, 10, 50, 100, 150 and 200 nm. $\Delta\lambda$ = 10 nm was chosen for the analytical technique development because of its high sensitivity and narrower spectrum in the excitation wavelength range λ_{ex} = 250–700 nm. The spectral data were collected using Fluoroescence software and OrginPro 6.0 software was used for further data analysis. The calibration curves were plotted taking SFS intensity vs. concentration of protein sample. The linear regression fits were made by applying OrginPro 6.0 software.

3. Results and discussion

3.1. Characterization by conventional fluorescence

The binding affinity of a fluorescence probe with album plays an important role during protein estimation because binding between probe and protein molecules could alter the fluorescence of the probe. All probes are not sensitive to such change in fluorescence upon binding with protein molecules, so identifying right kinds of fluorescence probe in this regard can be crucial. The fluorescence emission spectrum (figure not shown) of chrysene in the phosphate buffer showed a peak at 404 nm whereas the excitation maximum or absorption maximum was found to be located at 267 nm. Addition of BSA shifted the emission maximum of chrysene towards the blue, peak at 354 nm whereas a small change (red shift) was obtained in the excitation spectrum, peak at 270–275 nm upon addition of BSA. In the presence of HSA the fluorescence emission maximum of chrysene were found to be in the range 367–387 nm and in the presence of γ-G it was in the range 341–345 nm. Shifting of emission/exication maximum of chrysene in the presence of protein molecules is due to hydrophobic nature of the probe which strongly binds with the hydrophobic pockets present in the albumin and thus, altering chrysene's fluorescence properties. Upon increasing the concentration of BSA/HSA/γ-G the fluorescence intensity increased appreciably with a slight variation in emission maximum as statistically more number of chrysene could now bind to the large number of hydrophobic pockets of protein available due to increase in protein population.

Photo-bleaching or photochemical stability of probe molecule is another concern during fluorescence based methods. β-CD, CB6 or CB7 are common host molecules and their cooperative binding with guest molecule such as fluorescence probe in the presence of protein not only improves the photochemical stability but also increases the fluorescence quantum yield (Bhasikuttan et al., 2007). Our hypothesis was apart from above benefits, such host molecules may help to reduce the close contact between hydrophoic fluorescence probe (guest molecule) and other polar ions that directly

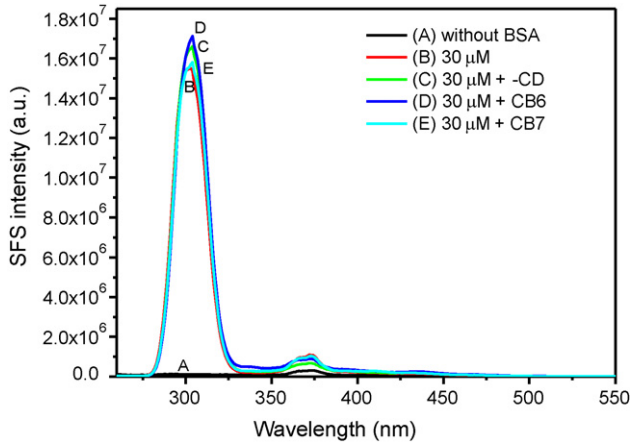

Fig. 1. Synchronous fluorescence spectra of chrysene in the absence and presence of BSA along with various host molecules, (A) without BSA, (B) 30 μM BSA, (C) 30 μM BSA with β-cyclodextrin, (D) 30 μM BSA with cucurbitu[6]uril, and (E) 30 μM BSA with cucurbit[7]uril.

quenches/enhances the fluorescence of the probe molecule in the sample solution. Therefore, β-CD, CB6 or CB7 were chosen as host molecule during albumin determination by using chrysene as fluorescence probe. The fluorescence emission and excitation spectra of chrysene did not show any remarkable change in the spectral position and shape in the presence of 10 μM of β-CD, CB6 or CB7 except a marginal increase in fluorescence quantum yield indicating a weak host–guest complex, as expected for a hydrophobic guest molecule (chrysene) in a more polar environment of the host pocket of CB6 or CB7 in this concentration range. The change in fluorescence intensity of chrysene in the absence and presence of β-CD, CB6 or CB7 was also minimal when BSA/HSA/γ-G was present in the solution. The inner core of such host molecules is more hydrophobic than the outer region. Hydrophobic probe molecule, chrysene, would prefer to sit in the inner core of host molecule forming host–guest complex changing its fluorescence characteristic. However, such complex formation also depends on the radius of inner core and size of the guest molecule.

3.2. Optimization of SFS method

SFS method was applied for the determination of albumin protein because a narrower, sharp and higher sensitive spectrum could be obtained during analytical estimation beside simplicity of the measuring tools. The synchronous fluorescence spectra are narrower and more symmetric than the fluorescence spectra ($\lambda_{em}/\lambda_{ex}$ = 354/267 nm), so the synchronous fluorescence method was used to determine protein. Among various wavelength interval ($\Delta\lambda$) tested during albumin measurement, $\Delta\lambda$ = 10 nm gave most sensitivity and a sharper peak to identify albumin binding to chrysene. When $\Delta\lambda$ = 10 nm, the synchronous fluorescence maximum of chrysene is at 374 nm and in the presence of BSA a new sharp peak having higher intensity appears at 296 nm. Synchronous fluorescence spectra of chrysene in the absence and presence of BSA is depicted in Fig. 1. The SFS intensity of chrysene at 296 nm increased dramatically when chrysene bound to BSA whereas addition of host molecules, β-cyclodextrin, cucurbitu[6]uril or, cucurbit[7]uril in the presence of protein molecule had little influence on the SFS intensity of chrysene at this wavelength region.

As shown in Fig. 2, upon increasing the concentration of BSA/HSA/γ-G the synchronous fluorescence intensity increased dramatically with slight change in synchronous fluorescence maximum (λ_{SFS}^{max}). The interaction of BSA with chrysene was expected to have high binding affinity due to hydrophobic nature of chrysene in buffer sample (phosphate buffer). It was also easier to identify binding of chrysene with protein using synchronous fluorescence spectra due to appearance of a new peak. Cooperative binding of chrysene with β-CD, CB6 and CB7 in the presence of BSA did not alter remarkably the position of peak in the emission, excitation and synchronous fluorescence spectra. However, with increasing concentration of BSA, the SFS intensity consistently increased as expected with population of BSA. Similarly in the presence of HSA the synchronous fluorescence maximum of chrysene was found at 294 nm, whereas in the presence of γ-G it was in the range 301–309 nm. The synchronous fluorescence intensity of chrysene increased continuously (in case of HSA at 294 nm and for γ-G in the range 301–309 nm) with increasing protein concentration. The trends obtained for cooperative binding of chrysene with β-CD, CB6 and CB7 in the presence of various concentrations of HSA or γ-G were similar to as observed for BSA.

3.3. Calibration graphs and sensitivity

Scanning the excitation and emission monochromators simultaneously the synchronous fluorescence spectra can be obtained in which the synchronous fluorescence intensity is given as:

$$I_{SFS} = kCdE_{EX}(\lambda_{em})E_{EM}(\lambda_{ex} + \Delta\lambda)$$

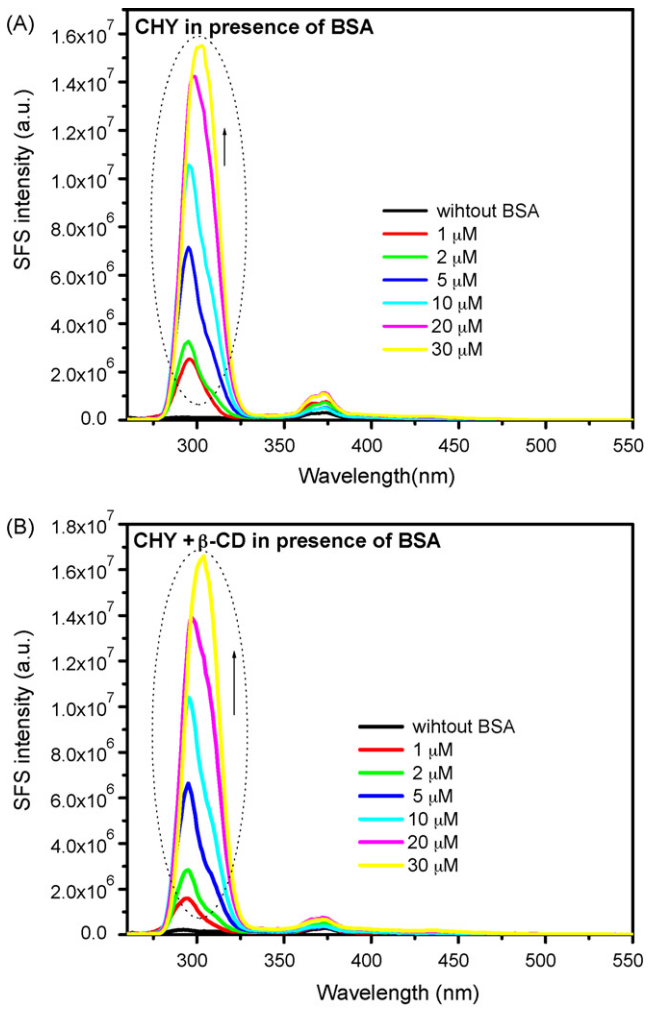

Fig. 2. Synchronous fluorescence spectra of chrysene in the absence and presence of various concentration of BSA, (A) without any host molecule and (B) with β-cyclodextrin.

Table 1
Analytical parameters for the determination of albumin proteins.

Protein	Host	Dynamic range (μM)	Regression equation	r	LOD (μM)
BSA	–	0.01–0.3	$I_{SFS} = 118{,}426.55 + 1.94E^6C$	0.991	0.005
		0.1–3.0	$I_{SFS} = 344{,}013.01 + 1.60E^6C$	0.994	
		1.0–20.0	$I_{SFS} = 1.13{,}481E^6 + 53524.094C$	0.997	
	β-CD	0.01–0.3	$I_{SFS} = 132{,}989.47 + 2.37E^6C$	0.990	0.005
		0.1–3.0	$I_{SFS} = 517{,}441.84 + 155{,}561.95C$	0.996	
		1.0–20.0	$I_{SFS} = 399{,}194.89 + 559{,}006.01C$	0.998	
	CB6	0.01–0.3	$I_{SFS} = 195{,}627.58 + 2.97859E^6C$	0.997	0.005
		0.1–3.0	$I_{SFS} = 448{,}241.69 + 156{,}335.76C$	0.998	
		1.0–20.0	$I_{SFS} = 839{,}568.99 + 564{,}493.54C$	0.996	
	CB7	0.01–0.3	$I_{SFS} = 124{,}425.23 + 2.48E^6C$	0.998	0.005
HSA	–	1.0–30.0	$I_{SFS} = 159{,}924.07 + 135{,}168.43C$	0.998	0.5
	β-CD	1.0–30.0	$I_{SFS} = 89{,}524.87 + 143{,}014.31C$	0.999	0.5
	CB6	1.0–30.0	$I_{SFS} = 154{,}436.73 + 140{,}102.54C$	0.998	0.5
	CB7	1.0–30.0	$I_{SFS} = 143{,}483.25 + 119{,}768.92C$	0.998	0.5
γ-G	–	0.01–0.3	$I_{SFS} = 281{,}111.28 + 2.25E^6C$	0.997	0.005
		0.1–3.0	$I_{SFS} = 252{,}722.74 + 1.94E^6C$	0.997	
		1.0–30.0	$I_{SFS} = 1.74E^6 + 566{,}999.44C$	0.973	
	β-CD	0.01–0.3	$I_{SFS} = 89{,}921.31 + 4.75E^6C$	0.981	0.005
		0.1–3.0	$I_{SFS} = 282{,}410.18 + 1.83E^6C$	0.995	
		1.0–30.0	$I_{SFS} = 1.46E^6 + 575{,}124.27C$	0.977	
	CB6	0.01–0.3	$I_{SFS} = 274{,}746.73 + 2.01E^6C$	0.998	0.005
		0.1–3.0	$I_{SFS} = 288{,}304.15 + 1.90E^6C$	0.995	
		1.0–30.0	$I_{SFS} = 1.92E^6 + 603{,}023.98C$	0.979	
	CB7	0.01–0.3	$I_{SFS} = 204{,}721.09 + 2.03E^6C$	0.998	0.005
		0.1–3.0	$I_{SFS} = 246{,}646.71 + 1.77E^6C$	0.998	
		1.0–30.0	$I_{SFS} = 2.26E^6 + 558{,}664.93C$	0.970	

where I_{SFS} is the relative intensity of synchronous fluorescence, E_{EX} is the excitation function at the given excitation wavelength ($\lambda_{ex} = \lambda_{em} - \Delta\lambda$), E_{EM} is the normal emission function at the corresponding emission wavelength ($\lambda_{em} = \lambda_{ex} + \Delta\lambda$), C is the analytical concentration, d is the thickness of the sample cell, and k is the characteristic constant comprising of the "instrumental geometry factor" and related parameters. Since the relationship of the synchronous fluorescence intensity (I_{SFS}) and the concentration of protein should follow the I_{SFS} equation, the I_{SFS} should be in direct proportion to the concentration of albumin. The calibration graphs for BSA/HSA/γ-G were constructed from the results obtained under the optimal conditions. These equations, along with limits of detection were obtained according to the general procedure. The proportional correlation of the enhancement intensity of synchronous fluorescence with the concentration of BSA is in the range 0.01–0.3 μM. The linear regression data for the calibration curves for estimation of BSA, HSA, and γ-G by chrysene as sensor with/without host molecules such as β-CD, CB6 or CB7 are summarized in Table 1 and shown in Fig. 3 (see also Supplement figure). It was found that in the presence of host molecules the SFS intensity improved slightly but the change was not dramatic. Such enhancement can be attributed to macrocyclic host molecule that imposes a structural confinement of the included chromophoric guest molecule by increasing its thermal and photochemical stability, and isolate it from the bulk water, which together improves its fluorescence properties (Bortulus and Monti, 1996; Ikeda and Shinkai, 1997; Wagner et al., 2001; Maïrquez and Nau, 2001). The calibration curve for estimation of BSA by applying chrysene as fluorescence sensing material could be useful in a wide range of concentration from 0.01 to 30 μM, however, to specify utility of the curves in different concentration ranges, three linear dynamic ranges in between 0.01 and 0.3 μM, 0.1 and 3.0 μM and 1.0 and 20.0 μM were plotted, the data for linear regressions are supplied in Table 1. The linear dynamic range attained in this study was not only found to be broad but also the limit of detection acquired for BSA estimation using current SFS based biosensing technique

Table 2
Tests for the interference of coexisting substances along with chrysene and BSA in the absence and presence of β-CD.

Interfering metal ions	β-CD	Ratio of SFS intensity in the absence and presence of interfering ions	Change in SFS intensity before and after addition of metal ion	Remark on addition of β-CD
$Al(NO_3)_3$	Absent	0.99	−1%	No change
	Present	1.01	+1%	
$CaCl_2$	Absent	0.85	−15%	Improvement
	Present	1.05	+5%	
$CoCl_2$	Absent	1.35	+35%	Improvement
	Present	1.08	+8%	
$FeSO_4$	Absent	0.79	−21%	Improvement
	Present	1.09	+9%	
$Fe(NO_3)_3$	Absent	0.68	−32%	Improvement
	Present	1.03	+3%	
$HgNO_3$	Absent	1.24	+24%	Improvement
	Present	1.07	+7%	
$ZnSO_4$	Absent	0.89	−11%	Improvement
	Present	0.99	−1%	

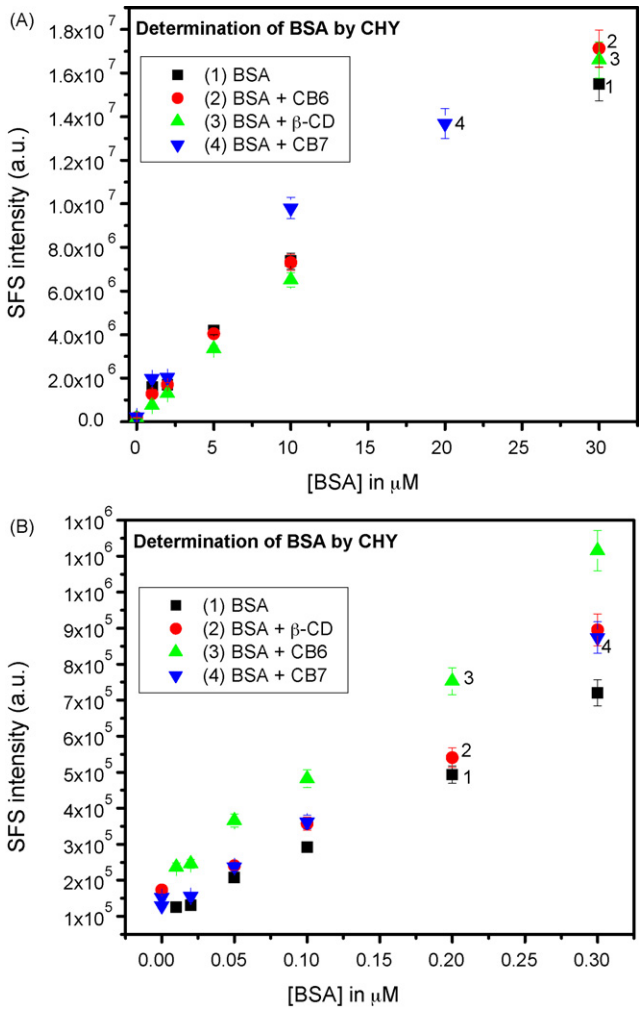

Fig. 3. Change in SFS intensity of chrysene in the absence and presence of various concentration of BSA (A and B). The relative standard deviation during the measurements was less than 10%.

was 0.005 μM which is much more sensitive than other reported method (Wang et al., 2002). Similarly for HSA and γ-G, the dynamic ranges, linear regression data and limit of detection as gathered in Table 1.

3.4. Interference of coexisting foreign substances

Metal ions are well known as fluorescence quenchers and enhancers (Holmes et al., 1993; Meier and Schubert, 2005). The fluorescence of PAHs is often quenched or enhanced by metal ions (Aguirre et al., 1987; Masuhara et al., 1984; Nakamura et al., 1983; Tucker et al., 1992). It is expected that host molecule such as β-CD, CB6 or CB7 would diminish close contact between the hydrophoic guest molecule and other polar ions that directly quenches/enhances the fluorescence of the proble molecule in the sample solution. Therefore, the influence of foreign coexisting substances in the absence and presence of β-CD was tested by selecting various metal ions that directly influence fluorescence of chrysene. The ratio of SFS intensity in the absence and presence of interfering ions and change in SFS intensity before and after addition of metal ion are presented in Table 2. It was found that in the absence of host molecule, Al (NO_3) had little impact during protein estimation by using chrysene as guest molecule, where as $CaCl_2$, $CoCl_2$, $FeSO_4$, $Fe(NO_3)_3$, $HgNO_3$, $ZnSO_4$ could influence the change in SFS intensity up to 11–35%. However, in the presence of host molecule such as β-CD the interference from metal ions such as $CaCl_2$, $CoCl_2$, $FeSO_4$, $Fe(NO_3)_3$, $HgNO_3$ and $ZnSO_4$ reduced notably. For example, the change in SFS intensity before and after addition of $Fe(NO_3)_3$ reduced from −32% without β-CD to merely 3% with β-CD. Similarly reduction for $CoCl_2$ was from 35% to 8%, for $HgNO_3$ from 24% to 7%, for $CaCl_2$ from −15% to 5%, for $FeSO_4$ from −21% to 9% and for $ZnSO_4$ from −11% to −1%. Among all the metal ions studied maximum improvement in interference was observed for $Fe(NO_3)$.

4. Conclusion

The synchronous fluorescence biosensor has high sensitivity for the determination of albumin, particularly for BSA and γ-globunin, when chrysene is used as a fluorescence probe and β-CD, CB6 or CB7 as host molecule. Appearance of separate SFS peak in the lower (blue) wavelength region upon binding of chrysene with albumins can easily indentify albumin present in the sample. Thus, unlike conventional fluorescence emission or excitation spectral measurement, SFS becomes a unique tool to identify the presence of albumin in the biological sample. Cooperative binding of chrysene in a supramolecular host–protein assembly improves the interference from coexisting foreign metal ions. SFS measurement could be done much faster than conventional separation based method for protein estimation and fluorescence process gives higher sensitivity which could be further improved by confining the probe molecule in a host system. Therefore, apart from reducing interference, applying the proposed method higher sensitivity, simplicity and rapidity could be achieved. Further studies in this field will open up the way to applications of cooperative binding of probe in a supramoleccular host-bioanalyte assembly in biosensor and analytical biochemistry.

Acknowledgements

Financial support provided by Lebanese National Council for Scientific Research (LNCSR) and American University of Beirut, Lebanon through the University Research Board (URB) long-term Faculty Development grant to carry out this work is greatly acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2009.09.041.

References

Abraham, W.J., 2002. J. Incl. Phenom. Macrocycl. Chem. 43, 159.
Aguirre, M.J., Lissi, E.A., Olea, A.F., 1987. J. Photochem. 36 (2), 177.
Babic, N., Larson, T.S., Grebe, S.K., Turner, S.T., Kumar, P., Singh, R.J., 2006. Clin. Chem. 52 (11), 2155.
Baczynskyj, L., Bronson, G.E., Kubiak, T.M., 1994. Rapid Commun. Mass Spectrom. 8 (3), 280.
Bhasikuttan, A.C., Mohanty, J., Nau, W.M., Pal, H., 2007. Angew. Chem. Int. Ed. 46, 4120.
Bortulus, P., Monti, S., 1996. Adv. Photochem. 21, 1.
Busby, D.E., Bakris, G.L., 2004. J. Clin. Hypertens. 6 (11 (Suppl. 3)), 8.
Chan, O.T., Herold, D.A., 2006. Clin. Chem. 52 (11), 2141.
Comper, W.D., Osicka, T.M., 2005. Adv. Chronic Kidney Dis. 12 (2), 170.
Diamond, D., McKervey, M.A., 1996. Chem. Soc. Rev. 25, 15.
Hirayama, K., Akashi, S., Furuya, M., Fukuhara, K., 1990. Biochem. Biophys. Res. Commun. 14 (2), 639, 173.
Hennig, A., Bakirci, H., Nau, W.M., 2007. Nat. Methods 4, 629.
Holmes, A.S., Suhling, K., Birch, D.J., 1993. Biophys. Chem. 48 (2), 193.
Ikeda, A., Shinkai, S., 1997. Chem. Rev. 97, 1713.
Kleinpeter, M.A., 2007. Cardiometab. Syndr. 2 (1), 63.
Koner, A.L., Nau, W.M., 2007. Supramol. Chem. 19 (1–2), 55.
Marquez, C., Hudgins, R.R., Nau, W.M., 2004. J. Am. Chem. Soc. 126, 5806.
Maĭrquez, C., Nau, W.M., 2001. Angew. Chem. Int. Ed. 40, 3155.
Masuhara, H., Shioyama, H., Saito, T., Hamada, K., Yasoshima, S., Mataga, N., 1984. J. Phys. Chem. 88 (24), 5868.

Meier, M.A.R., Schubert, U.S., 2005. Chem. Commun. (36), 4610 (Cambridge, United Kingdom).
Mohanty, J., Bhasikuttan, A.C., Nau, W.M., Pal, H., 2006. J. Phys. Chem. B 110, 5132.
Mohanty, J., Nau, W.M., 2004. Photochem. Photobiol. Sci. 3, 1026.
Mohanty, J., Pal, H., Ray, A.K., Kumar, S., Nau, W.M., 2008. ChemPhysChem 8, 54.
Mohanty, J., Nau, W.M., 2005. Angew. Chem. Int. Ed. 44, 3750.
Nakamura, T., Kira, A., Imamura, M., 1983. J. Phys. Chem. 87 (16), 3122.
Nau, W.M., Mohanty, J., 2005. Intern. J. Photoenerg. 7, 133.
Patra, D., Ghaddar, T.H., 2009. Talanta 77, 1549.
Patra, D., Mishra, A.K., 2000a. Anal. Lett. 33, 2293.
Patra, D., Mishra, A.K., 2000b. Analyst 125, 1383.
Patra, D., Mishra, A.K., 2001. Talanta 53, 783.
Patra, D., Mishra, A.K., 2002a. Trends Anal. Chem. 21, 787.
Patra, D., Mishra, A.K., 2002b. Anal. Bioanal. Chem. 374, 304.
Patra, D., Mishra, A.K., 2006. In: Grimes, C.A., Dickey, E.C., Pishko, M.V. (Eds.), Encyclopedia of Sensors, vol. 2. American Scientific Publishers, California, USA, p. 139.
Rankin, M.A., Wagner, B.D., 2004. Supramol. Chem. 16, 513.
Ruhn, P.F., Taylor, J.D., Hage, D.S., 1994. Anal Chem. 66 (23), 4265.
Shaikh, M., Singh, P.K., Mohanty, J., Nau, W.M., Pal, H., 2008. Photochem. Photobiol. Sci. 6, 408.
Singh, R., Crow, F.W., Babic, N., Lutz, W.H., Lieske, J.C., Larson, T.S., Kumar, R., 2007. Clin. Chem. 53 (3), 540.
Toto, R.D., 2004. J. Clin. Hypertens. 6 (11 (Suppl. 3)), 2 (Greenwich).
Tucker, S.A., Acree Jr., W.E., Fetzer, J.C., Jacob, J., 1992. Polycyclic Aromat. Compd. 3 (1), 1.
Wagner, B.D., Stojanovic, N., Day, A.I., Blanch, R.J.J., 2003. Phys. Chem. B 107, 10741.
Wagner, B.D., Fitzpatrick, S.J., Gill, M.A., MacRae, A.I., Stojanovic, N., 2001. Can. J. Chem. 79, 1101.
Wang, L.-Y., Zhou, Y.-Y., Wang, L., Zhu, C.-Q., Li, Y.-X., Gao, F., 2002. Anal. Chim. Acta 466, 87.
Walcher, W., Franze, T., Weller, M.G., Pöschl, U., Huber, C.G., 2003. J. Proteome Res. 2 (5), 534.
Zhang, G., Fan, H., Xu, C., Bao, H., Yang, P., 2003. Anal. Biochem. 313 (2), 327.
Zhang, X., Gramlich, G., Wang, X., Nau, W.M., 2002. J. Am. Chem. Soc. 124, 254.