Research Journal of Chemical Sciences ISSN 2231-606X
Vol. **4(12),** 93-97, December **(2014)** *Res. J. Chem. Sci.*

Fluorescence Quenching Studies and Binding Interactions of β-Casein and Therapeutic Chemicals Mediated by Ag Nanoparticles and Cu Nanoparticles

Vasumathi V. and Santhanalakshmi J.*
Department of Physical Chemistry, University of Madras, Guindy Campus, Chennai -600025, Tamilnadu, INDIA

Available online at: www.isca.in, www.isca.me
Received 29th November 2014, revised 3rd December 2014, accepted 16th December 2014

Abstract

Fluorescence quenching studies on the milk protein β Casein (BC) with certain chemotherapeutics acting as quenchers are studied. Around six organics such as urea (U), Thiourea(TU), Guanidine Hydrochloride (GHCl), 4-Amino antipyrine (AP), Paracetamol (P) and Guaiacol (G) are chosen as quenchers and found sensitive to addition of metal nanoparticles such as Silver nanoparticles (Ag nps) and Copper nanoparticles (Cu nps) respectively. The asynthesised Ag nps and Cu nps in this work possessed the mean sizes as 12±1nm and 18±1nm as found from HRTEM measurements. The extent of fluorescence quenching was found to be more in presence of metal nanoparticles than in absence of metal nanoparticles. The binding constant (K_B) and the number of binding sites (n) are obtained from Stern – Volmer plot and double reciprocal plot methods. The data indicate that the mediating capacity of Ag nps is higher than the Cu nps systems. This may be attributed to the smaller sized Ag nps than the Cu nps. The trend observed in the interaction between various organics and BC has been found to be U > TU > GHCl > AP > P > G. This trend remains the same even in the presence of metal nanoparticles. The exhibited interacting activity of the chemicals is attributed to the difference in the interaction of surfacial hydrophilic groups in BC with organics studied here.

Keywords: Silver nanoparticles, copper nanoparticles, fluorescence quenching, β-casein, urea, thiourea, paracetamol, guaiacol, guanidine hcl, 4-aminoantipyrine.

Introduction

β Casein (BC) is as natural milk protein abundantly available, highly amphiphilic and is a fluorophore with characteristic fluorescence at 350 nm. In life systems, milk protein metabolism can be subjected to the action of different substances such as diamines, aromatics, phenols, acids etc., and studies pertaining to the related effects of interactions between the milk proteins and additives may be expected to bring out the information on the extended applications of milk protein to biological[1-3].

Recently, the application of milk protein as drug delivery systems has provided novel results and the concept of effective oral drug delivery by BC has emerged out.

The amphiphilic nature of BC structure, suggest the entrapment and successful delivery hydrophobic chemotherapeutics through hydrophobic interactions[4-5]. Since, BC is a significant fluorophore, analysis of the effect of quenchers and the extent of quenching result can be used to monitor the binding interactions between the milk protein and selected quencher. The availability of suitable and effective oral drug delivery agents would make significant contribution in reducing the painful complications associated in administering such chemicals in life systems. The goal of the current study is to develop BC as an interactive chemotherapeutic delivery system on certain selected chemicals when the chemotherapeutics are used as quenchers, towards the fluorescence quenching of BC, then the binding interaction constant (K_B) and number of binding sites (n) can be precisely evaluated using well proven methods like Stern-Volmer plot and Klotz-Klotz double reciprocal plots.

The quenchers chosen are Urea (U), 4-Amino antipyrine (AP), Guanidine Hydrochloride (GHCl), Paracetamol (P), Thiourea (TU) and Guaiacol(G). Each of the chemicals possess unique structural property and usages in many biochemical processes. Transition metals and metal ions in solutions are well known to act as fluorescence quenchers and also to interact strongly with milk proteins. With the emergence of metal nanoparticles in biological applications due to nano size and unique properties in optical, electrical , magnetic and bio sensing devices, the metal nanoparticles of Ag and Cu can be chosen as quenchers on the BC fluorescence [6-8].

Incidentally, the chemotherapeutics chosen as quenchers on the BC, fluorescence are expected to exhibit altered interactions in the presence of metal nanoparticles due to the changes in the extense of hydrophobic and hydrophilic interactions with BC. These quenchers posses strongly interacting functional groups such as carbonyl, amino and phenolics. Addition of metal nanoparticles would effectively alter the interactions between BC. Such interactions can be detected to be favourable or non favourable based on the binding parameters, when measured in the presence and absence of metal nanoparticles. Such results aid to derive information on the role of hydrophilic and

hydrophobic forces between BC and chemotherapeutics in the presence of metal nanoparticles.

Material and Methods

β-casein, purchased from sigma (99% purity) was used. The quenchers such as 4-Amino antipyrine, Guanidine Hydrochloride, Paracetamol, Thiourea and Guaiacol were purchased from Aldrich. The metal nanoparticles are prepared with analar grades of CTAB (Cetyl Trimethyl Ammonium Bromide) (purity > 99%), Copper nitrate, Silver nitrate and Sodium borohydride from Lobachemie, India. All the chemicals were used without further purification. Aqueous solutions are prepared with double distilled water distilled in all glass assembly.

Synthesis of Ag nanoparticles and Cu nanoparticles: One pot batch reactor type method involving addition of 2 mL of 2% by weight of CTAB solution to 10 mL of 1mM solution of metal salt precursor with continuous stirring at 25^0C was maintained. To this mixture, 5 mL of 0.1M freshly prepared sodium borohydride solution was added drop wise under continuous stirring for 6 hours with slight heating up to 40^0C. Appearance of lemon yellow coloured solution and wine red coloured solution indicate the formation of Ag nps and Cu nps respectively. The nanoparticles suspensions are stored in N_2 for further use[9-12].

Nanoparticles size measurements: A drop of the nanoparticles solution (5 µl) was placed on formavar coated Cu grid (200 meshes). The sample was allowed to stand for 1 minute and excess solution is removed by filter paper and dried in air. The samples were size characterised using Philips Technai -12 Transmission Electron Microscope operated at 120 KV[13-14].

Steady state fluorescence: Experiments with BC solutions are measured using luminescence spectrometer (RF-5301, Japan, Shimadzu) equipped with thermostated water circulation and PC interface. The excitation and emission slits are fixed at 3.0nm and 1.5nm respectively and the excitation wavelength is set as 295nm. The emission spectra are collected between 300nm - 500nm.

To 10µl of BC solution, 50µl of Cu nps solution was added and the emission spectra of BC before and after the addition of metal nps are measured[15-18]. To this mixture, quencher solution (0.1mM) was added in small aliquots (1 µl) and the emission intensities are recorded. Adopting similar conditions, BC emissions before and after the addition of quencher solutions are recorded first, followed by the small aliquot addition of metal nanoparticles. By reversing the addition sequence, changes in the emission are recorded which reflect the interactions between metal nps mediators and BC with and without the quencher.

Binding studies: The intensity of the characteristic broad emission band of BC at 350nm decreases markedly with increasing concentrations of the additive quencher, which act as quenchers. The fluorescence quenching data are used to obtain binding parameters including binding constant and the number of binding site values. Assuming fluorescence quenching to be a dynamic quenching process, the apparent bimolecular quenching rate constant (K_q) was calculated using Stern-Volmer equation method with and without quenchers[19-24].

$$\frac{I_0}{I} = 1 + K_q \tau_0 [Q]$$

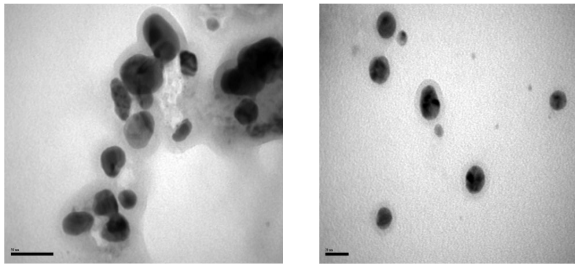
Where I_0 and I are the fluorescence intensities in the absence and presence of quencher, [Q] is the concentration of quencher, K_{SV} is the Stern–Volmer dynamic quenching constant, K_q is the bimolecular quenching rate constant and τ_0 is the average bimolecular lifetime in the absence of quencher (τ_0) which is evaluated as 3.30 nanoseconds. From the linear plot of I_0/I versus quencher concentration, the K_{SV} value is obtained from the slope. K_q value is determined from slope and τ_0 values. Regarding the number of binding sites (n) between BC and quencher, the double reciprocal plots was constructed based on the following equation

$$\frac{1}{r} = \frac{1}{n} + \frac{K_B}{n}\frac{1}{[Q]}$$

Where 'r' equals to number of moles of quencher bound per mole of the protein, BC. The reciprocal of the intercept produces the 'n' value. Using slope and n values, K_B is determined[25-28].

Results and Discussion

Mean sizes of Ag nps and Cu nps: The CTAB capped Ag nps and Cu nps in the assynthesised conditions are size characterised using HRTEM technique. In figure-1 the HRTEM photographs are given. The mean nanosizes of Ag nps and Cu nps are determined to be 12±1 nm and 18±1 nm respectively. Around 300 particles are counted and Poisson's distribution was applied.



I ii

Figure-1
HRTEM of i) Ag nanoparticles and ii) Cu nanoparticles

Quenching Studies: In figure-2, the fluorescence emission spectra of BC in free form and in presence of quenchers along with Ag nps and Cu nps are given. Under constant composition conditions, the decrease in fluorescence intensity of BC in the presence of quenchers gets altered when Ag nps and Cu nps are

added. In all cases, the quenching ability was found to increase in the presence of Ag nps and Cu nps. The intensity of fluorescence of BC decreased drastically when the sequence of addition is metal nanoparticles and the quenchers compared to quenchers and the addition to metal nanoparticles. This is due to the metal nanoparticles interacting strongly with BC exposing the hydrophilic groups in such a way favouring the interactions with quenchers. Also, based on the extent of decrease in the fluorescence intensity, a trend on the strength of interaction between the quenchers and BC may be evaluated.

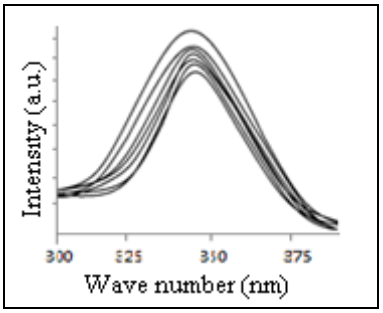


Figure-2
Fluorescence spectra of (i) β-casein, ii) β casein + Thiourea, iii) β casein + Thiourea + Cu nps, iv) β casein + Thiourea + Ag nps, v) β casein + Urea, vi) β casein + Urea + Cu nps, vii) β casein + Urea + Ag nps

Binding constant and binding sites (K_B, n): The quenching characters of quenchers are studied from the Stern Volmer (S-V) plots shown in figure-3, constructed in the presence and absence of Ag nps and Cu nps which are given in Figure 3. Best fit S-V plots are found. The values of Stern-Volmer constant (K_{SV}) for each of the quencher system are given in table-1. The K_{SV} value is the outcome of the combination of biomolecular quenching constant (K_q) and the lifetime of the fluorophore in the absence of quencher (τ_0). The K_B values are also determined in the absence of metal nanoparticles combining the different K_B values evaluated in the absence and presence of metal nanoparticles. The role of metal nanoparticles altering the hydrophobic and hydrophilic interactions with quenchers and BC can be inferred. In Figure 4, the double reciprocal plots for BC with various chemotherapeutics are presented for both in the presence and absence of Cu nps and Ag nps.

Based on the K_B values, it is found that Ag nps interact strongly with BC more efficiently than Cu nps. This effect causes the quenchers to quench the fluorescence of BC to a greater extent in the presence of metal nanoparticles than in the absence of the same. Also, the K_B values of quenchers determined in the absence of metal nanoparticles are found to be lower than the K_B values of quenchers in the presence of metal nanoparticles. Among Ag nps and Cu nps, the mediating effects of Cu nps on BC and the quenchers are found less stronger than that cast by Ag nps which, results in the K_B values estimated in the presence of Ag nps.

The observed trend in the quenching interaction of different quenchers applied on BC, based on the respective K_B values is U > TU > GHCl > AP > P > G. These compounds have more than one functional group and the overall activity is the result of the combination of the various functional groups. U exhibits more basic nature than TU and rest of the compounds. TU is found to be more acidic, hydrophilic and less dissociative than U. GHCl is well known to interact with protein and exhibits less protein denaturing property. This may be due to some possible hydrophobic interactions with casein. However, surfacial hydrophilicity of the protein is more prevalent in the binding interactions in aqueous media.

In case of AP and G, there is no significant change in the binding interactions in the presence of metal nanoparticles as seen in the nearly constant values of K_B in the presence of Ag nps and Cu nps. Regarding the number of binding sites (n) possible for the quenchers on the hydrophilic surfaces of BC in the absence of metal nanoparticles nearly 1:1 interactions are seen. AP, P and G exhibit two molecular binding of quenchers per BC molecules. In the presence of Ag nps and Cu nps, 'n' is almost doubled for all quencher system studied. With the enhanced K_B values in the presence of metal nanoparticles, it can be certainly inferred that Ag nps and Cu nps act as favourable mediators for the quenchers chosen in the present work to interact and bind strongly to the BC compared to BC in free state.

Table-1
The binding parameters (K_B, n) and K_{SV} values of the quenchers interaction with BC in the absence and in the presence of Cu nps and Ag nps at 25^0C

Quencher	Binding Parameters								
	Absence of nps			Presence of Ag nps			Presence of Cu nps		
	K_{sv}	K_B	n	K_{sv}	K_B	n	K_{sv}	K_B	n
Urea	1.07E+00	9.81E-01	1.04	1.19E+00	1.59E+00	1.75	1.18E+00	1.49E+00	1.69
Thiourea	2.11E+00	3.23E-01	1.05	3.38E+00	8.53E-01	1.65	2.53E+00	7.88E-01	1.41
Guanidine Hydrochloride	5.27E+01	2.33E-02	1.20	5.55E+01	4.06E-02	2.09	5.35E+01	3.90E-02	1.97
4-Amino antipyrine	1.50E+04	2.11E-04	1.5	1.55E+04	2.51E-04	1.61	1.55E+04	2.11E-04	1.52
Paracetamol	1.18E+05	3.45E-05	2.0	1.47E+05	4.41E-05	2.12	1.28E+05	4.20E-05	2.00
Guaiacol	1.18E+05	3.10E-05	1.85	1.44E+05	3.19E-05	1.93	1.20E+05	3.16E-05	1.85

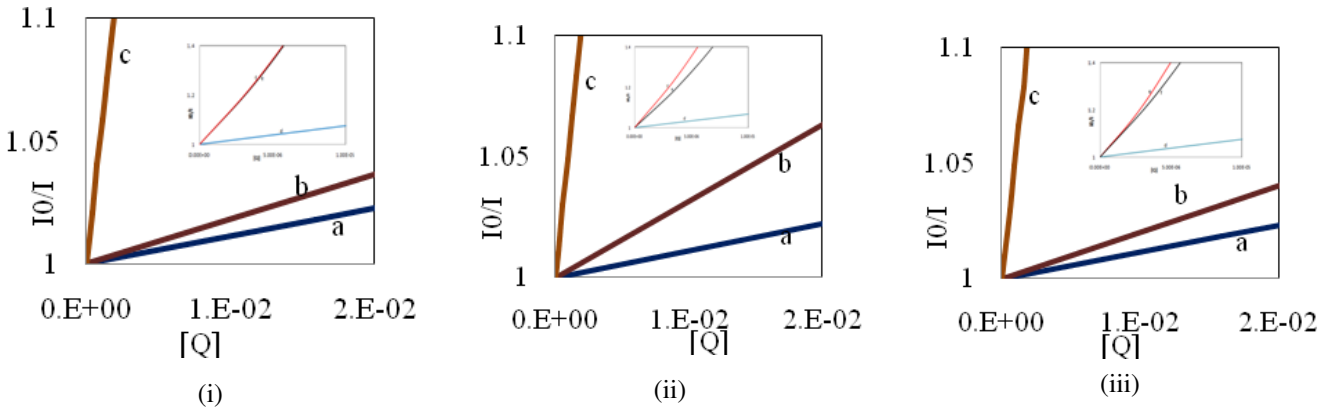


Figure-3
Stern-Volmer plots of β casein in the i) absence of nanoparticles ii) presence of Ag nps, iii) presence of Cu nps. Quenchers: a - Urea, b - Thiourea, c - Guanidine Hydrochloride, d - 4-Aminoantipyrine e – Paracetamol and f – Guaiacol

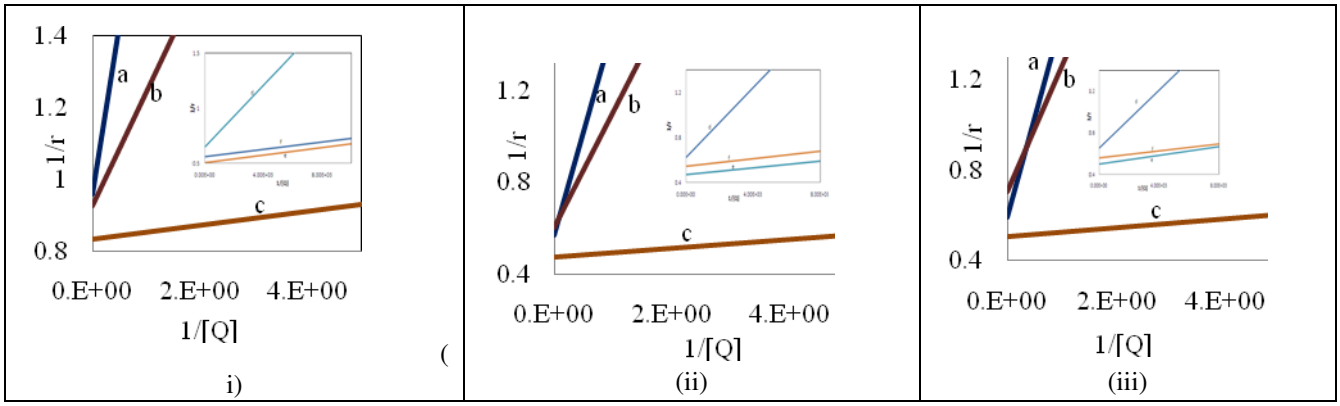


Figure-4
Double reciprocal plots of β-casein in the i) absence of nanoparticles ii) presence of Ag nps and iii) presence of Cu nps. Quenchers: a - Urea, b - Thiourea, c - Guanidine Hydrochloride, d - 4-Aminoantipyrine, e – Paracetamol and f – Guaiacol

Conclusions

The binding interactions of the milk protein BC with chemotherapeutics like urea, thiourea and related compounds were determined in the presence of Ag nps and Cu nps which are prepared in the present work. The additives are used as quenchers on the fluorescence intensity exhibited by BC. Applying Stern-Volmer plot and double reciprocal plot method, the K_B and n are determined for the six chemotherapeutics interactions with BC in the presence of metal nanoparticles. Ag nanoparticles produced effective changes in the interaction constant than the Cu nps. The trend in the interaction extent with BC among the six organic chemicals is found to be Urea > Thiourea > Guanidine Hydrochloride > 4-Aminoantipyrine > Paracetamol > Guaiacol. The higher basicity of urea and higher acidity of other chemicals along with thiourea have exhibited this type of interaction trend. Also, presence of metal nanoparticles mediated the exposure of surfacial hydrophilic groups of BC to facilitate such interactions. The results prove the favourable interactions of such chemicals with BC motivating further studies on the ability of the milk protein to act as an efficient and drug delivery system.

Acknowledgements

The authors wish to thank National Centre for Nanoscience and Nanotechnology, University of Madras for the TEM results.

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