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Fluorescence Quenching Studies on the Interactions Between β-Casein and α – Amino acids Mediated by Cu Nanoparticles and Ag Nanoparticles

V. Vasumathi and J.Santhanalakshmi*

Department of Physical Chemistry, University of Madras, Guindy Campus, Chennai -600025, Tamilnadu, India.

Abstract: Interactions of the milk protein β-casein (BC) and α-amino acids (AA) are studied using BC fluorescence quenching measurements. Seven AA such as L-Glycine (Gly), L-Serine (Ser), L-Lysine (Lys), L-Histidine (His), L-Glutamic acid (Glu), L-Aspartic acid (Asp) and DL-Valine (Val) are used as fluorescence quenchers and BC fluorescence at 350nm was found to be sensitive to AA. AA and metal nanoparticles are found to interact strongly. Hence, the interactions between BC and AA are studied by fluorescence quenching method in the absence and in the presence of silver nanoparticles (Ag nps) and copper nanoparticles (Cu nps). Cu nps and Ag nps are synthesised using CTAB as the capping agent and size characterised using TEM measurements. The sizes of Cu nps and Ag nps are found to be 18±1nm and 12±1nm respectively. The fluorescence data are analysed adopting Stern-Volmer plot and double reciprocal plot methods. The binding constant (K_B) and the number of binding sites (n) values are determined. The data indicate that Ag nps caused enhanced interactions between BC and AA more effectively than the Cu nps which is more than in the absence of nanoparticles. This effect may be due to the smaller sized Ag nps than the Cu nps. Also, the trend on the interaction with BC, among the seven AA has been found to be Ser > His > Lys > Gly > Glu > Val > Asp. The results testify the enhanced interaction of AA with BC in the presence of Cu nps and Ag nps.
Key words: Nanoparticles, Fluorescence quenching, β-Caesin, α-amino acids.

1. Introduction

Recently, considerable research attention has been focussed on the studies related to the interactions of bio-active macromolecules (proteins) and nanoparticles (nps) of metals because such results are expected to provide useful and basic information on the extended applications of metal nps in biological applications. Especially, metal nps of gold, silver, platinum, copper, nickel etc., have been utilised for this purpose [1-3]. Milk protein is one of the most abundant proteins with nutritional values and found sensitive to the nature of the interacting materials. β-casein (BC) is a milk protein which is applied significantly in all milk based preparations bearing pharmaceutical, therapeutical, nutritional and biological significances [4-6]. β-casein exhibits fluorescence due to the characteristic fluorophores such as tryptophan and other amino acids present in it. The intensity of the β-casein fluorescence (350nm) has been found to be sensitive to the presence of fluorescence quenchers such as transition metal ions, organic amines, phenolic, carboxylic etc., substrates.

In the present work, we have carried out physicochemical investigations on the interactions of β-casein with seven essential α-amino acids such as L-Glycine(Gly), L-Serine(Ser), L-Lysine(Lys), L-Histidine(His), L –Glutamic acid(Glu), L-Aspartic acid(Asp) and DL-Valine(Val) using fluorescence measurements. With the emergence of extended applications of transition metal nps, in the studies involving proteins, organic substrates, polymers etc., fluorescence quenching studies and the results from it are available only in smaller numbers. Metal and metal ions in solutions are well proven to act as fluorescence quenchers. These materials interact strongly with the fluorophores. In the present work, application of transition metal nps such as copper nps(Cu nps) and silver nps(Ag nps) as fluorescence quenchers of β-casein fluorescence in the presence and absence of

α-amino acids. β-casein and α-amino acids interact well and such interactions cause changes in the fluorescence behaviour of β-casein. Also such interactions are altered in the presence of metal nps. Hence, the metal nps are expected to mediate the interactions between β-casein and α-amino acids. Adopting the fluorescence quenching studies, the interaction parameters between BC and several α-amino acids can be determined effectively utilising the mediating behaviours of Ag nps and Cu nps. For this purpose Cu nps and Ag nps are prepared using CTAB (cetyl trimethyl ammonium bromide), a cationic surfactant as the stabiliser. Here, the interactions between some of the amino acids exhibited favourable interactions, while Ser and Glu exhibited less favourable interactions with BC. Presence of nps mediator increased the interactions more favourably which is seen from the higher binding constant values (K_B) and the number of binding sites (n). These results can be used to derive information on the role of structure based activity relationships between the AA and BC macro molecules at the molecular level.

2. Experimental

Chemicals: β-casein was purchased from sigma (99%) and all the seven α-amino acids were purchased from Aldrich. CTAB (purity > 99%), analar grades of Copper nitrate, Silver nitrate and Sodium borohydride were purchased from Lobachemie, India. All the chemicals are used as it is.

Method:

BC aqueous solutions are prepared as 1 mg per 30 ml. The BC solutions are filtered through a Millipore filter with 300nm pore size and kept at 25^0C for 12h before use [7-10].

Synthesis of nanoparticles: Cu nps and Ag nps are prepared with the copper nitrate and silver nitrate as the precursors. 10 ml of 1mM solutions of the metal salt solution was taken in a round bottom flask and stirred with 2 ml of 2% by weight of CTAB solution and thermostatted at 25^0C. To this mixture, 5ml of 0.1 molar freshly prepared sodium borohydride is added drop wise and continuously stirred for 6 hrs at 40^0C. Lemon yellow coloured solution resulted for Ag nps while wine red coloured solution resulted for Cu nps [11-12].

Size characterisation: A drop of the nps 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, 50µl of Cu nps solution was added and the emission spectra of BC before and after the addition of metal nps are measured. To this mixture, α-amino acid 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 AA solutions are recorded first, followed by the small aliquot addition of metal nps. 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 AA.

Binding studies: The intensity of the characteristic broad emission band of BC at 350nm decreases markedly with increasing concentrations of the additive AA, 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 [15-20].

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

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 which is evaluated as (τ_0) 3.30

nanoseconds. From the linearplot 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 α-amino acids, 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 [21-23].

3. Results and Discussion

3.1 Mean sizes of Ag nps and Cu nps

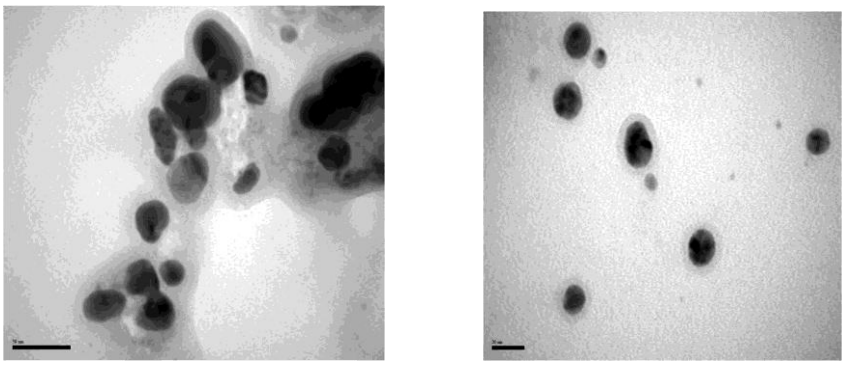

Figure 1: HRTEM of i) Ag nanoparticles and ii) Cu nanoparticles

The as-synthesised Ag nps and Cu nps as mentioned in the experimental are size characterised using HRTEM measurements. In Fig.1, it may be seen that the morphology of nps are nearly mono disperse spheres in both the cases and applying Poisson's distribution , the mean nanosizes of the Cu nps and Ag nps are found to be 18±1nm and 12±1nm respectively, considering 300 particles count.

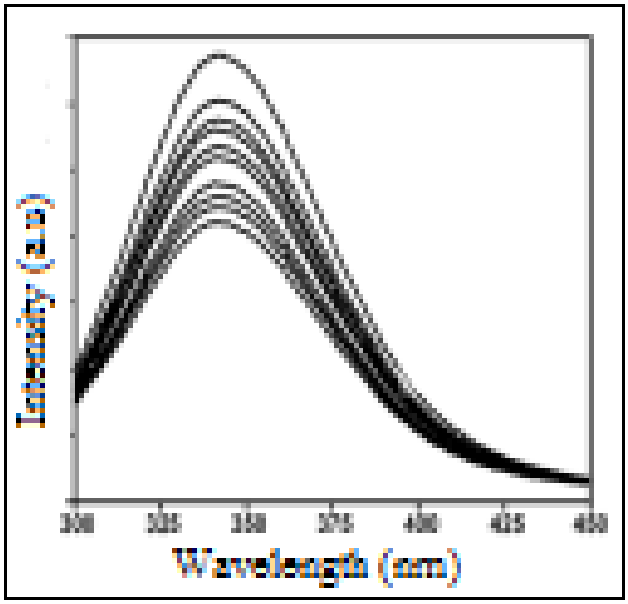

Figure 2: Fluorescence spectra of β-casein (i) in the pure and in the presence of ii)Histidine iii)Cu nps iv)Ag nps v)Histdine + Cu nps vi)Histidine + Ag nps vii)Cu nps + Histidine viii)Ag nps + Histidine ix)Cu nps + Serine x)Ag nps + Serine

The fluorescence emission spectra of BC in presence and absence of α-amino acids, Cu nps and Ag nps are given in Fig.2. Under constant composition conditions, the fluorescence intensity of BC in the absence of

any additive (I_0) seems to be greater than in presence of α-amino acids, Cu nps and Ag nps which are added separately (I). Therefore, the chosen AA are considered as quenchers. The decrease in the I_0 of BC is higher when the quenchers are added in sequence as Cu nps / Ag nps and α-amino acids compared to the decrease in I_0 of BC when AA is added prior to Cu nps/Ag nps. Therefore, the interaction among BC, Cu nps/Ag nps and AA may be stronger compared to BC-α-amino acids, BC-Cu nps/Ag nps and BC -α-amino acids-Cu nps/Ag nps.

3.2 Binding constant and binding sites (K_B, n)

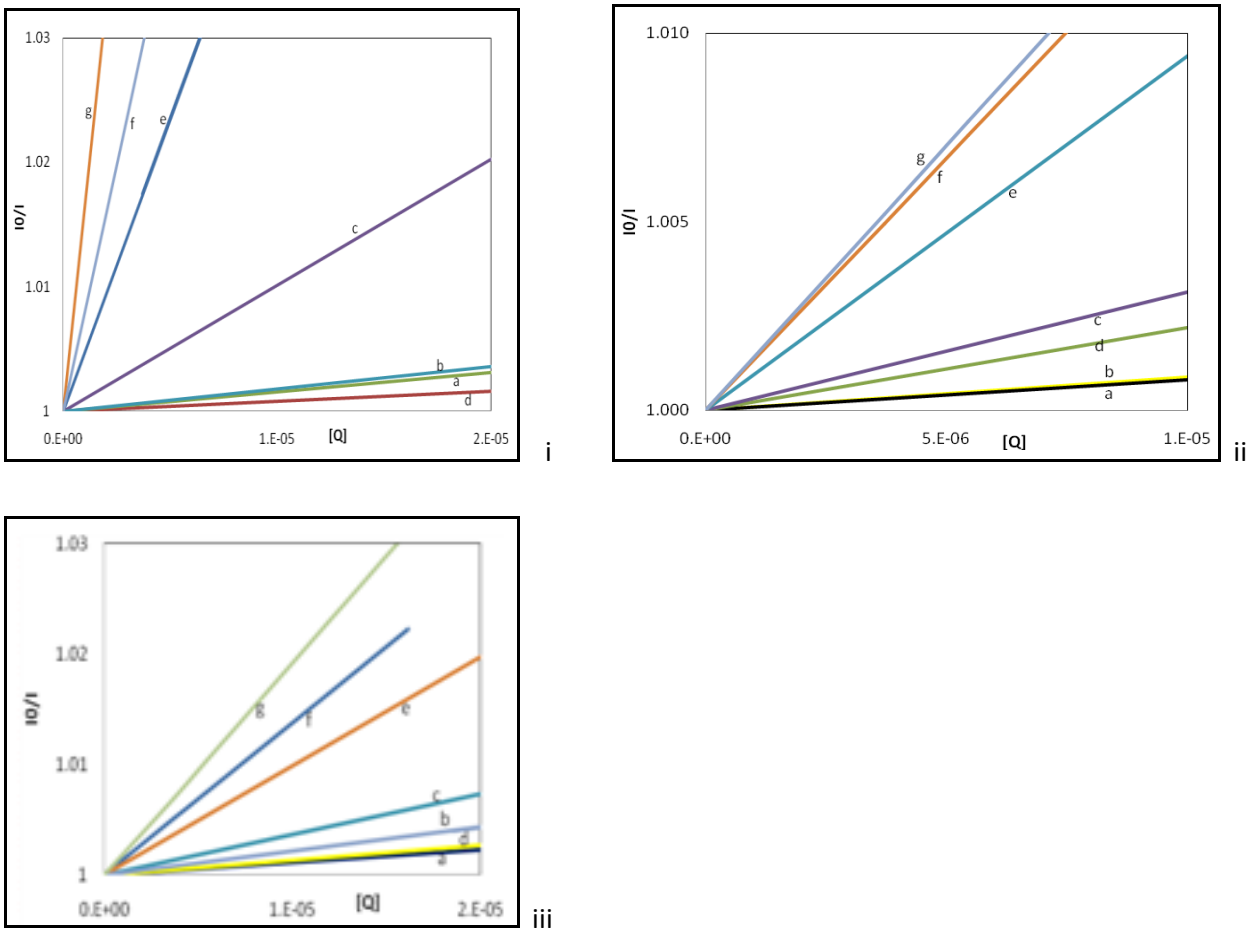

Figure 3: Stern-Volmer plots of β-casein with i) α-amino acids as quenchers ii) same in presence of Cu nps and iii) same in presence of Ag nps ; a) Serine; b) Histidine; c) Glycine; d) Lysine; e) Glutamic acid; f) Valine and g) Aspartic acid

In Fig.3, the Stern-Volmer plots of BC with various α-amino acids, in the absence and in the presence of Cu nps and Ag nps are given. Linear plots obeying the Stern-Volmer equation are found. The values of Stern-Volmer constants (K_{SV}) are given in Table 1. Also linear Stern-Volmer plots indicate the presence of interaction between a single class of fluorophore equally accessible to the quenchers. The K_{SV} value is the result of the combination of the values of bimolecular quenching constant, K_q and the lifetime of the fluorophore in the absence of any quencher τ_0. Hence, considering the following equilibrium,

$$\text{Protein} + n\ \text{Quencher} \underset{}{\overset{K_B}{\rightleftharpoons}} [\text{Protein} - \text{Quencher}_n],$$

where Protein is BC, Quencher is α-amino acid /Cu nps/Ag nps, K_B is the value of binding constant and n is the number of binding sites.

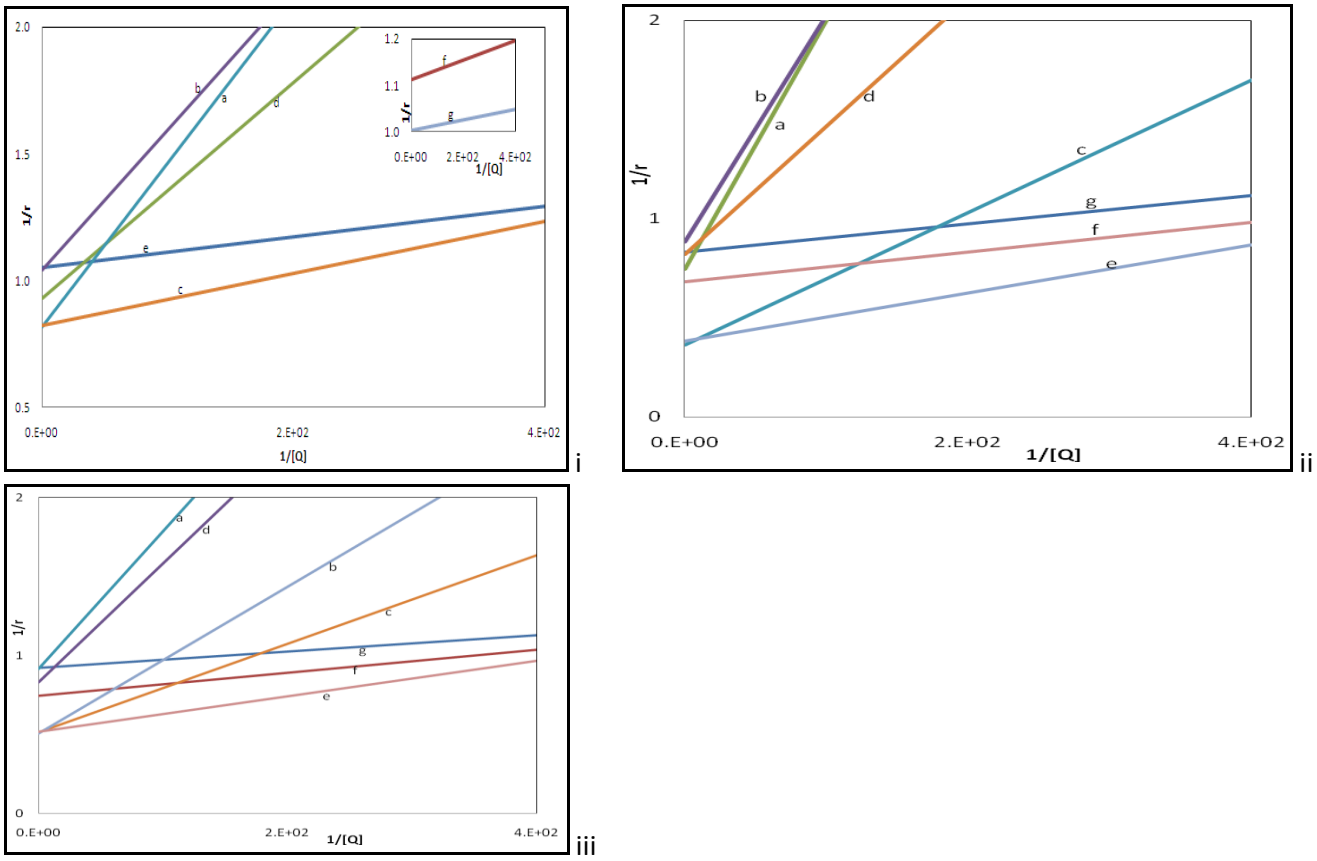

Figure 4: Double reciprocal plots of β-casein with α-amino acids in the i) absence ii) presence of Cu nps and iii) Ag nps. a) Serine; b) Histidine; c) Glycine; d) Lysine; e)Glutamic acid; f) Valine and g) Aspartic acid.

In Fig.4, the double reciprocal plots of BC with α-amino acids, in the absence and in the presence of Cu nps and Ag nps are given. The values of binding parameters are given in Table 1.

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

Quencher (Amino Acid)		Binding Parameters								
		Absence of nps			Presence of Ag nps			Presence of Cu nps		
a	Serine	K_{sv}	K_B	n	K_{sv}	K_B	n	K_{sv}	K_B	n
b	Histidine	1.57E+02	7.90E-03	1.22	8.09E+01	1.68E-02	1.34	1.15E+02	9.52E-03	1.09
c	Glycine	1.80E+02	5.35E-03	0.97	8.78E+01	1.29E-02	1.13	2.19E+02	9.18E-03	1.98
d	Lysine	1.02E+03	1.25E-03	1.21	3.16E+02	9.22E-03	2.76	3.70E+02	5.48E-03	1.95
e	Glutamic acid	2.38E+02	4.59E-03	1.08	1.98E+02	1.07E-02	2.02	1.36E+02	9.06E-03	1.2
f	Valine	4.69E+03	5.81E-04	0.96	9.51E+02	3.18E-03	2.62	1.02E+03	2.18E-03	1.94
g	Aspartic acid	1.53E+03	1.90E-04	0.89	4.88E+02	1.10E-03	1.46	5.07E+02	9.78E-04	1.34

The values of Stern-Volmer constant (K_{SV}) and binding constant (K_B) show similar trend on the interaction between BC with different AA in presence of Cu nps and Ag nps. Based on the K_B values, Ag nps seem to interact with BC more efficiently than the Cu nps and both the nano metal particles interact with BC more efficiently than the interactions of BC with individual α-amino acids. In aqueous environment, Ag nps bind to the surface hydrophilic groups of BC more strongly than the Cu nps, and this is reflected in the K_B values of BC-Cu nps and BC–Ag nps systems. Regarding α-amino acids, along with the weak hydrophilic interactions, penetration effect of α- amino acids into the hydrophobic core of the BC protein molecule due to the strong hydrophobic interactions are possible. The K_B values of BC with different AA are found lower than the K_B values of BC-Ag nps and BC-Cu nps which indicate that hydrophilic interactions are stronger than the

hydrophobic interactions with BC. It is well reported that, several AA interact well with copper and silver metal / metal ion components [24]. Therefore, considering the K_B values, and the extents of fluorescence quenching, it is revealed that Cu nps and Ag nps are good mediators in enhancing interactions of AA with BC. Also, the mediating capacities of Ag nps are found more efficient than the Cu nps between BC and AA.

The observed trend in the interaction of BC among the seven AA studied separately and based on the K_B values, is Serine > Lysine > Glycine > Glutamic acid > Valine > Aspartic acid.

Similar trend was observed in the presence of Cu nps and Ag nps as well. AA interacting with BC through both hydrophobic and hydrophilic interactions show higher K_B values than the AA interacting through hydrophilic interactions only.

Regarding the number of binding sites (n), higher K_B values are seen for n > 1.0, because, increase in the n value strengthens the interactions and thus K_B values are increased. In the presence of Cu nps and Ag nps the n values are considerably exceeded than 1.0, since Cu nps and Ag nps are act as mediators enabling both the hydrophilic and hydrophobic interactions.

4. Conclusions

Fluorescence of β-casein protein was found to be quenched in the presence of α-amino acids and Cu nps / Ag nps when added separately. Adopting Stern-Volmer plot and double reciprocal plot methods, the number of binding sites(n), K_B and K_{SV} constant values are determined for seven amino acids selectively chosen based on the quenching characteristics. In the presence of Cu nps and Ag nps, K_{SV}, K_B and n values are different. Based on the K_B values, the trend in the interaction with BC among the seven amino acids is studied and found to be Ser > His > Lys > Gly > Glu > Val > Asp. In presence of Cu nps and Ag nps the same trend was observed considering K_B values. In presence of Ag nps, the values of n exceed 1.0. This may be attributed to the surfacial reactivity of Ag nps with individual AA. The results clearly indicate the dominating role of Cu nps and Ag nps as mediators in the interactions between BC and AA. The AA interacting with BC through hydrophilic and hydrophobic interactions exhibit higher K_B values than those AA interacting with BC through hydrophilic interactions only.

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