

Kinetic and thermodynamic study of beta-Boswellic acid interaction with Tau protein investigated by surface plasmon resonance and molecular modeling methods

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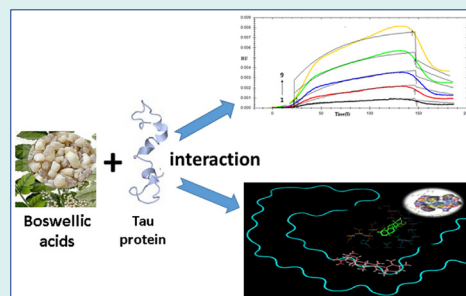
Abstract

Introduction: Beta-Boswellic acid (BBA) is a pentacyclic terpene which has been obtained from frankincense and its beneficial effects on neurodegenerative disorders such as Alzheimer's disease (AD) have been addressed.

Methods: In the present study, thermodynamic and kinetic aspects of BBA interaction with Tau protein as one of the important proteins involved in AD in the absence and presence of glucose has been investigated using surface plasmon resonance (SPR) method. Tau protein was immobilized onto the carboxy methyl dextran chip and its binding interactions with BBA were studied at physiological pH at various temperatures. Glucose interference with these interactions was also investigated.

Results: Results showed that BBA forms a stable complex with Tau ($K_D=8.45 \times 10^{-7}$ M) at 298 K. Molecular modeling analysis showed a hydrophobic interaction between BBA and HVPGGG segment of R₂ and R₄ repeated domains of Tau.

Conclusion: The binding affinity increased by temperature enhancement, while it decreased significantly in the presence of glucose. Both association and dissociation of the BBA-Tau complex were accompanied with an entropic activation barrier; however, positive enthalpy and entropy changes revealed that hydrophobic bonding is the main force involved in the interaction.



Introduction

Neurodegenerative diseases (NDDs) are traditionally defined as disorders with selective and progressive loss of structure or function of neurons. Alzheimer's disease (AD) is one of the most common types of NDDs which is characterized by the progressive loss of memory and other cognitive functions.¹ Cognitive failure in the AD is the result of different co-pathologic interactions.² Different studies have suggested a central role for proteins in the development of AD. Accumulation of senile plaques leading to extracellular β -amyloid (β) and intracellular neurofibrillary tangles (NFTs; pathologically aggregated Tau) are among the major hallmarks in the brain of the AD

patients.³ Attempts to find protein-based biomarkers for diagnostic aims and development of various therapeutic strategies were the results of improved understanding of protein role in AD.⁴ In this regard, the development of vaccines against Tau protein has been explored.⁵

As an intrinsically disordered protein (IDP), Tau protein possesses a random coil (in solution) structure in physiologic conditions;³ while in the AD, their aggregation leads to PHF-insoluble Tau (mostly β -sheet structure) (Fig. 1A-C).⁶ This aggregation is a result of various physiologic factors such as oxidative stress and imbalance actions of kinases and phosphatases.^{5,7,8}

Investigation of small molecules with the ability of

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binding to Tau protein or Tau physiologic function-related enzymes/drug targets have emerged as an interesting field in AD research⁹. Among these are natural compounds. Herbal remedies and food-derived agents as nutraceuticals have become more popular in the treatment of NDDs and the benefits derived from these phytotherapeutics have been very promising.¹⁰

Frankincense referred to as Oleo-gum resin which is extracted from the trees of the genus *Boswellia* of Burseraceae family and is obtained after an incision into the bark of the tree.¹¹ Beta-Boswellic acid (BBA) (Fig. 1D) is the most abundant compound in the extract of resins.¹² Yassin et al¹³ reported the protective and therapeutic effects of *Boswellia Serrata* extract on the AD in rats (a decrease in the levels of NFTs in the affected regions of the brain). Additionally, in an *in vitro* study, it was shown that BBA is able to enhance neurite outgrowth, branching, and polymerization dynamics of tubulin.¹⁴

In the present study, the binding interaction of BBA with Tau protein and its kinetic and thermodynamic parameters were investigated using surface plasmon resonance (SPR) technique. The interaction was also studied in the presence of different concentrations of glucose. Glucose is the main source of energy for the brain.¹⁵ Glucose deprivation can occur in aging and NDDs.¹⁶ Clinical investigations have highlighted a biological link between reduced brain glucose metabolism and AD.¹⁷ Diabetes and insulin resistance are strong risk factors for cognitive decline and AD.^{18,19} The effect of glucose on tau aggregation both via interfering in Tau phosphorylation,²⁰ Tau cleavage and Tau glycation^{17,21} have been considered as probable mechanisms in crosstalk between AD and diabetes.²¹

In addition, the effect of BBA on Tau stability and the secondary structure was studied by the application of CD measurements. Moreover, details of the interaction mechanism were investigated by the application of molecular docking and protein mapping studies. To the

best of our knowledge, no similar study has been reported.

Materials and Methods

Materials

Tau protein solution (in phosphate Buffer 50 mM) was purchased from biochemistry faculty of Shahid Beheshti University of Tehran. Its purity was checked by the application of the SDS-PAGE method²² and its concentration was measured by Bradford total protein assay method.²³ Tau aliquots were stored at -70°C until analysis. Before analysis, Tau was transferred to -20°C for 12 hours and then gently thawed at room temperature.

BBA (purity >95%) was purchased from Sigma-Aldrich (Germany). BBA stock solution (1×10^{-3} M) was prepared by dissolving the proper amount of it in HPLC grade methanol (Scharlau) and stored in dark glass at 4°C . The amount of methanol in all samples was <1%.

Sodium hydroxide (NaOH), N-hydroxysuccinimide (NHS), N-ethyl- N'- (3-diethylaminopropyl) carbodiimide (EDC), PBS (Phosphate-Buffered Saline) tablets and ethanolamine were purchased from Sigma-Aldrich (Germany). All these ingredients were of analytical grade. All solutions were diluted to the required volume with PBS buffer (pH 7.4), prepared from double-distilled water (prepared daily in Lab).

SPR measurements

A multi-parameter SPR device (MP-SPR Navi 210A, BioNavis Ltd, Tampere-region, Finland) equipped with a Kretschman prism configuration and a goniometer was used for SPR measurements. A peristaltic pump equipped with 100×10^{-6} L sampler loops was applied to inject the mobile phases (PBS, pH 7.4) with the flow rate of 30×10^{-6} L/min to dual flow channels. The experiments were carried out in fixed-angle mode. Sensor temperature varied between 298-310 K. A laser with a wavelength of 670 nm was used as a light source to excite the surface plasmon at the dielectric gold interface. The bulk effects were corrected by subtracting the response of a blank reference spot from the response of the ligand spots.²⁴ Different concentrations of BBA ($1-9 \times 10^{-6}$ M) were injected with a flow rate of 30×10^{-6} L.min⁻¹ for 2 minutes.

For minimizing the mass transport effect which leads to inaccurate data, the following approach was conducted in this study. First, the Tau molecule was immobilized using a low concentration Tau solution and the flow rate of test solutions for both kinetic and thermodynamic tests was set at a higher rate, i.e. 30×10^{-6} L.min⁻¹.

Carboxymethyl dextran (CMD) chips (Bionavis company, Finland) were used to immobilize Tau protein. These sensor chips were designed to allow detailed quantitative studies of small organic molecules, proteins interaction kinetics, and affinity. The ligands (Tau) were bounded to the sensor chip surface via carboxyl moieties on the dextran.

To obtain a stable baseline, PBS buffer (pH 7.4) was run

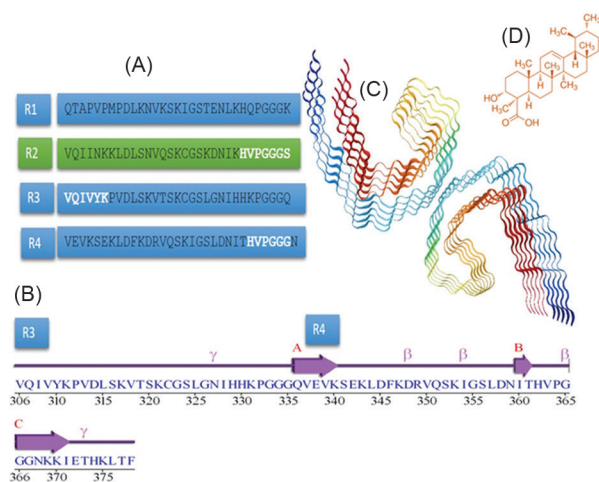


Fig. 1. (A) Amino acid sequence of R₁–R₄ of Tau repeat domains (microtubule-binding domains), (B) A sequence alignment of R₃ and R₄ repeat domains, (C) Schematic illustration of Tau protein paired filaments, (D) Chemical structures of Beta Boswellic acid.

for 20 minutes onto the CMD chip. Then NaCl (1 M) and NaOH (0.1 M) was run for 5 minutes to both channels in order to sensor surface cleaning.

Tau immobilization was carried out using carbodiimide (amine coupling) strategy. The carboxylated surface of the cleaned chip was activated using a solution of EDC (0.2 M): NHS (0.05 M) (50/50 V/V) which was injected for 6 minutes and then rinsed with PBS buffer solution (pH 7.4) for 5 minutes. The surface activated slides were exposed to Tau solution ($0.25 \text{ mg}\cdot\text{mL}^{-1}$ with a flow rate of $8.0 \times 10^{-6} \text{ L}\cdot\text{min}^{-1}$ for 10 minutes). The non-specific bindings were prevented by the injection of ethanolamine-HCl (1.0 M) to the chip after Tau immobilization.²⁵

Various concentrations of BBA ($1\text{-}9 \times 10^{-6} \text{ M}$) in PBS buffer (pH 7.4) were injected onto the Tau immobilized CMD chip (Tau-CMD) for 2 minutes. To investigate the nonspecific binding of BBA to the applied chip, a blank chip (without immobilized Tau) was inserted in the reference flow cell.

Investigation of glucose effect on BBA-Tau binding

To investigate the glucose effect in BBA-Tau interaction, Tau-CMD was inserted in both flow cells. BBA was injected into one of the chips after running a fixed flow rate of glucose on both chips. Various concentrations of BBA ($1\text{-}9 \times 10^{-6} \text{ M}$) in PBS buffer (pH 7.4) in the absence and presence of three separate concentrations (2.5, 5.0, and $20 \times 10^{-3} \text{ M}$) of glucose were injected for 2 minutes.

Study of Tau conformation after complex formation

To study the conformational variation of Tau due to BBA interaction, circular dichroism (CD) spectroscopy was used. CD spectra of Tau protein solution ($20 \times 10^{-6} \text{ M}$) before and after the addition of BBA ($5.0 \times 10^{-6} \text{ M}$) was recorded using a Jasco J-810 CD spectrometer (Lakewood, NJ, USA) equipped with a 0.1 cm path length sample cell. Data were collected with an interval of 1 nm (scan speed of 20 nm/min at 298 K) in the wavelength range of 200-250 nm. The baseline was corrected according to the PBS buffer solution (pH 7.4). The average of two measurements was analyzed using CDNN 2.1 CD curve de-convolution software. This software uses a neural network approach for calculating secondary structure features of proteins.

Molecular modeling study of BBA-Tau interaction

Probable binding sites were investigated using an online small molecule protein mapping server FTMap. BBA was used as a probe ligand for a hairpin structure of Tau repeat domain. The suggested binding site along with a known aggregation driver motif of the R₃ domain (³⁰⁶VQIVYK³¹¹)²⁶ was further investigated using molecular docking studies. BBA was docked to the selected motifs of Tau tubulin-binding repeat region crystal structure (PDB code: 5O3L, <https://www.rcsb.org/structure/5O3L>) using the gold software. The applied crystal structure was obtained using cryo-electron microscopy method²⁷ from

the paired helical filament in the AD brain (Fig. 1C). We used chain A as a representative of suitable conformation for aggregation. Both protein and BBA structures were optimized prior to docking with MOE software.²⁸ A standard docking procedure was used to find suitable conformations. Accordingly, Goldscore scoring function analysis followed by a rescoring using ChemScore scoring function method was used for molecular docking studies. The results were viewed using LigandScout (<http://www.inteliligand.com/ligandscout/>) and Pymol software and the best conformation was selected based on the frequency and orientation of the BBA in the studied binding site, while docking score was used for final selection between best results.

Results

Tau immobilization

Tau adsorption onto the activated surface of the chip could be achieved through both covalent amide coupling and electrostatic bond formation. Below the isoelectric pH of Tau (8.96), the amino groups possess positive charges,²⁵ while in this pH, carboxylic groups of CMD have negative charges. Therefore, the electrostatic bond formation is possible which facilitates the Tau attachment to the CMD chip surface at pH 7.4.

When a new layer was formed on Au sensor chip (e.g. CMD), the resonance curves shifted to higher incident angles and became wider which is a result of the increase in the thickness of the adsorbed layer. Accordingly, it is possible to evaluate the immobilization process through monitoring the response unit (RU) before and after Tau immobilization.

RU of untreated CMD was 0.01 which increased significantly to 0.025 after surface activation with EDC/NHS and decreased to 0.01 following surface cleaning. After the immobilization of Tau on the chip surface, RU value increased to 0.05. The remaining activated carboxylic acid groups were deactivated using ethanolamine (Fig. 2A). The steepest slope in the sensorgram occurred during the binding of Tau to the chip surface. This steep falling slope was consequent with a shift in total internal reflection (TIR).²⁹ The angle shift of CMD chip surface in comparison with the Tau-CMD chip has been shown in Fig. 2B. The angle shifted from 0.079 to 0.102 after Tau immobilization. According to the results of the activation of CMD, Tau immobilization was performed properly.

BBA-Tau interaction studies

The real-time binding profiles of BBA ($1\text{-}9 \times 10^{-6} \text{ M}$) with Tau have been presented in Fig. 3A. At all BBA concentrations, the responses reached equilibrium. The RU increased due to the BBA concentration enhancement.

K_D , K_a and K_d values were obtained from fitting the equilibrium RU to a one-site binding model using Trace Drawer TM software. Kinetic properties of the binding procedure were produced using the SPR sensorgrams of

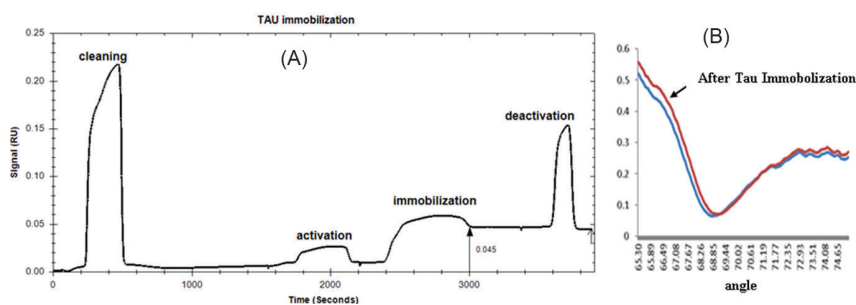


Fig. 2. (A) SPR sensogram of Tau immobilization on a CMD chip at PH 7.4 [Activation: activation of the CMD with a mixture of EDC/NHS (injection time: 6 min, flow rate: 30×10^{-6} L/min)- Immobilization: immobilization of 0.25 mg/mL Tau (injection time: 10 min, flow rate: 8×10^{-6} L/min)- Deactivation: deactivation of remaining reactive NHS esters with ethanolamine (injection time: 3 min, flow rate: 30×10^{-6} L/min)].

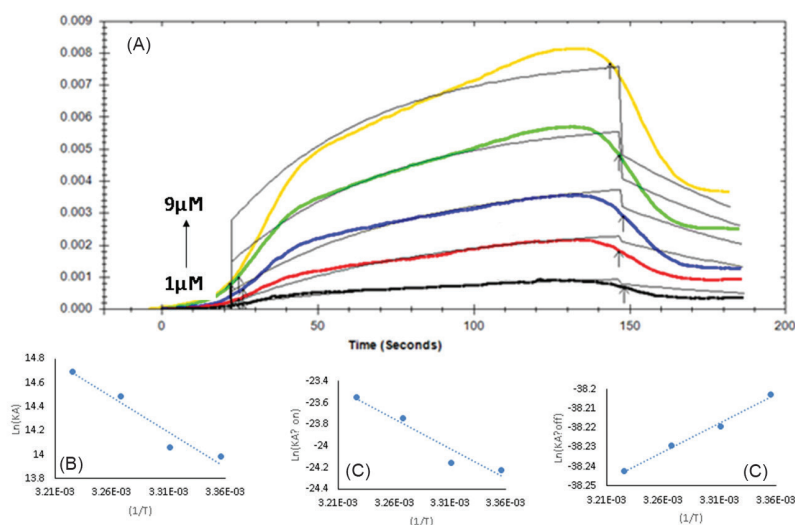


Fig. 3. (A) Sensogram BBA interaction with immobilized Tau (BBA $1-9 \times 10^{-6}$ M), (B) Van't Hoff plot for binding of BBA to the Tau protein in temperature range from 298 to 310 K. (C) and (D) Eyring plot of association and dissociation rate constants of BBA-Tau interaction, respectively.

various BBA concentrations as a function of time.³⁰

The value of K_D at 298 K was 8.45×10^{-6} M which shows the high affinity of BBA to Tau.³¹ Furthermore, K_a and K_d values were calculated as $1.18 \times 10^3 \text{ M}^{-1}\text{S}^{-1}$ and $1.0 \times 10^{-3} \text{ S}^{-1}$, respectively (Table 1). These results indicate that the rate constants have almost similar contribution in the binding of BBA molecules to Tau at 298 K.

Temperature dependence of the kinetics and affinity of the BBA interaction with Tau were investigated at various temperatures. The kinetic parameters were determined following injecting serially diluted concentrations of BBA in running buffer ($1-9 \times 10^{-6}$ M) to the Tau-CMD at various temperatures (298-310 K), and K_a , K_d and K_D values at different temperatures were calculated. The results are summarized in Table 1.

The results of fitting curves evaluated based on the theoretical one to one fitting model showed that RU values increase following increasing the BBA concentration (Fig. 3A). When these experiments were carried out at higher temperatures, it was found that the enhancement effect of BBA on RU values occurs at lower levels.

The K_D values decreased from 8.45×10^{-7} M at 298 K to

4.18×10^{-7} M at 310 K, suggesting that stronger bindings occur between Tau and BBA at higher temperatures. The association rate constant increased by 2.1 folds due to temperature enhancement from 298 K to 310 K, while the dissociation rate constant remained unchanged. This was well reflected in the corresponding K_D values. Therefore, the observed changes in the K_D values can be described by the increase in the association rate constant rather than a decrease in the dissociation rate constants.

Investigation of thermodynamic properties of BBA-Tau interaction

Van't Hoff plot (equation 1) was used to investigate the relationship between K_A and temperature.

Table 1. Kinetic data of BBA interaction with Tau

T (K)	$K_a(1/S) \times 10^{-3}$	$K_d(1/MS) \times 10^3$	$K_D(M) \times 10^{-7}$
298	1.00	1.18	8.45
302	0.99	1.28	7.81
306	1.00	1.95	5.13
310	1.00	2.40	4.18

$$\ln(K_A) = -\left(\frac{\Delta H^\circ}{RT}\right) + \left(\frac{\Delta S^\circ}{R}\right) \quad \text{Eq. (1)}$$

where ΔH° and ΔS° are standard enthalpy and entropy changes respectively, R is gas constant and T is the absolute temperature.³²

The standard ΔG° was calculated using the Gibbs free energy relationship (equation 2):

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad \text{Eq. (2)}$$

Binding enthalpy (ΔH), entropy (ΔS) and Gibbs energy change (ΔG) along with K_A values of the interaction were calculated using the binding constants at different temperatures (Table 2).

The results showed that there is a linear relationship between K_A and $1/T$ (Fig. 3B). This result indicates that ΔH° and ΔS° were practically independent of temperature in the studied temperature range. The negative slope indicates that the studied binding reaction was endothermic and an increase in the temperature results in a subsequent increase in K_A value.

Calculated ΔG° , ΔH° , and ΔS° values at 298 K were -3.46×10^4 , 4.86×10^4 Kcal/M and 2.79×10^2 Kcal/KM, respectively (Table 2).

The positive value of ΔH° indicates that the complex formation is an endothermic process and the standard enthalpy change upon the Tau-BBA complex formation is unfavorable. On the other hand, the positive ΔS° value indicates that entropy increases as the Tau-BBA complex is formed.

Negative ΔG° indicates that the binding reaction was thermodynamically favorable and proceeded spontaneously. It appears that the entropy and not the enthalpy drives the formation of the Tau-BBA complex. The spontaneous binding process is being driven by the entropy changes.

The transition state kinetics was studied using Eyring plot.³³ To obtain the activation of ΔH^{\ddagger} and ΔS^{\ddagger} , Eyring equation (equation 3) was used.

$$\ln(Kh/K_B T) = \Delta H^{\ddagger}/RT + \Delta S^{\ddagger}/R \quad \text{(Eq. 3)}$$

where K is the rate constant for the activated complex determined at temperature T (the absolute temperature)

in terms of ΔH^{\ddagger} and ΔS^{\ddagger} , K_B is the Boltzmann constant (1.38×10^{-23}), and h is the Planck constant (1.05×10^{-34}). Therefore, by measuring the rate constants ($K=K_a$ or K_d) at different temperatures, it is possible to obtain ΔH^{\ddagger} and ΔS^{\ddagger} values for the assembly of Tau-BBA (Fig. 3C and 3D).

The formation of the transition state for binding of BBA to Tau was characterized by an unfavorable enthalpy and entropy; whereas, the dissociation phase proceeded with a favorable enthalpy and unfavorable entropy. A positive value for ΔH^\ddagger in the association phase indicates that an unfavorable activation enthalpy (an activation enthalpy barrier) hinders the formation of the Tau-BBA transition state complex. On the other hand, there was a decrease in the enthalpic barrier to the transition state, facilitating the release of BBA from the Tau-BBA complex. Therefore, it seems that both association and dissociation of BBA and Tau are accompanied with an entropic activation barrier; however, the dissociation transition state of the complex is slightly compromised by a favorable enthalpy change in terms of the complex dissociation leading to the instability of the Tau-BBA complex.

Effects of glucose on Tau-BBA binding

BBA affinity to Tau was investigated in the presence of different concentrations of glucose. It was found that the presence of glucose interfered with the Tau-BBA complex formation in a way that affinity decreased significantly by the enhancement of glucose concentration (Table 3).

Conformational investigations

Fig. 4 shows far-UV CD spectra of Tau in the absence and presence of BBA. The double negative band around 208 ($\pi \rightarrow \pi^*$) and 222 nm ($n \rightarrow \pi^*$) and a positive band around 193 nm ($\pi \rightarrow \pi^*$) is characteristic for the typical α -helical structure for a pure α -helical structure,^{34, 35} while a negative band around 196 nm reveals random coil in the protein structure.

The mentioned features can be seen in Fig. 4, for not treated Tau protein in the studied condition in which both alpha-helix and random coil determinant bands are available. A similar CD curve for Tau protein was reported in previous studies. The complex formation between Tau and BBA leads to a variation in the Tau protein CD curve comparing with its unbound form in which one positive band around 193 appears which shows that the BBA

Table 2. Thermodynamic parameters for binding of the BBA to the Tau protein in PBS buffer pH 7.4

T (K)	Vant's Hoff		Eyring Plot (K_a)			Eyring Plot (K_d)			
	ΔH (Kcal/M) $\times 10^4$	ΔS (Kcal/KM) $\times 10^2$	$\Delta G^\circ \times 10^4$	ΔH (Kcal/M) $\times 10^4$	ΔS (Kcal/KM) $\times 10$	$\Delta G^\circ \times 10^4$	ΔH (Kcal/M) $\times 10^3$	ΔS (Kcal/KM) $\times 10^2$	$\Delta G^\circ \times 10^4$
298			-3.46	4.65	-4.63	6.03	-2.48	-3.27	9.48
302	4.86	2.79	-3.57			6.05			9.61
306			-3.68			6.07			9.74
310			-3.79			6.08			9.87

Table 3. Kinetic data of BBA-Tau interaction in the presence of glucose

Glucose (mM)	$k_d(1/S)\times 10^{-4}$	$k_a(1/MS)\times 10^3$	$K_D(M)\times 10^{-7}$
0	9.92*	2.2*	4.51*
2.5	9.96	1.89	5.26
5.0	9.86	1.39	7.10
20	9.98	1.24	8.02

*Derived from experiments in which reference cell consists of CMD-Tau.

binding leads to an increase in the α -helical structure of the Tau protein.³⁶

The calculated percentage of random coil and β -sheet in unbound Tau solution was 42.0% and 27.9%, respectively. After BBA (5×10^{-6} M) addition, the random coil and β -sheet was 40.7% and 24.9%, respectively. The α -helical content of Tau increased from 12.5% to 16.7% after BBA addition, which could be an indicator of the stability enhancement of Tau after complex formation with BBA.³⁷ It seems that BBA induces certain perturbations (increasing α -helical structure) on the Tau protein secondary structure which could affect the physiologic function of Tau.

Molecular modeling of the Tau-BBA interaction

Protein mapping revealed that there is a conserved hexapeptide in the R₄ domain of studied Tau filament (³⁶²HVPGGG³⁶⁷) (Fig. 1A), which possessed a probability of interaction with BBA (Fig. 5). In addition, the literature review shows that (³⁰⁶VQIVYK³¹¹) hexapeptide (R₃) is one of the most important motifs which is playing role in Tau aggregation.^{38,39} We investigated the molecular interaction of BBA with both hexapeptides using molecular docking studies. The results showed that the interaction pattern for the studied sites is different. These findings suggest that BBA can be situated near the (³⁶²HVPGGG³⁶⁷) segment

with a higher probability ($\Delta G=-21.24$ kcal.mol⁻¹ for the best score) (Fig. 5) in comparison to (³⁰⁶VQIVYK³¹¹) ($\Delta G=-17.46$ kcal.mol⁻¹ for the best score) considering the best scores, and the average ΔG for all conformations are -17.05 kcal.mol⁻¹ and -15.19 kcal.mol⁻¹, respectively for (³⁰⁶VQIVYK³¹¹) and (³⁶²HVPGGG³⁶⁷).

The molecular docking results showed that hydrophobic interactions are the main driving force for the binding which is consistent with the experimental results.

Discussion

As a widely used optical method for ligand interaction analysis, the SPR approach can be used for real-time monitoring of the small molecule-target affinity.⁴⁰ The association and/or dissociation of the studied analyte to the immobilized protein leads to changes in mass transfer and subsequently influence the refractive index at the surface layer.⁴¹ Variation in the refractive index causes changes in the SPR signal (RU). It is possible to determine both association and dissociation rate constants (k_a and k_d) separately. The equilibrium dissociation constant (binding affinity), K_D , can be calculated from the ratio of k_d to k_a for a given interaction. The k_a constant can be regarded as the number of BBA-Tau complex formed per second and the k_d is the fraction of the complex decays per second.⁴² according to the results the enhanced binding of BBA to Tau at higher temperatures could arise from a better diffusion of BBA molecules and its increased collision to the immobilized Tau at higher temperatures.

Previous evidence has identified that the positive ΔH° and ΔS° values are normally considered as the evidence for the hydrophobic interaction^{32,43}; it could be assumed that the binding of BBA to Tau occurs mainly through hydrophobic interactions. Additionally, the positive values

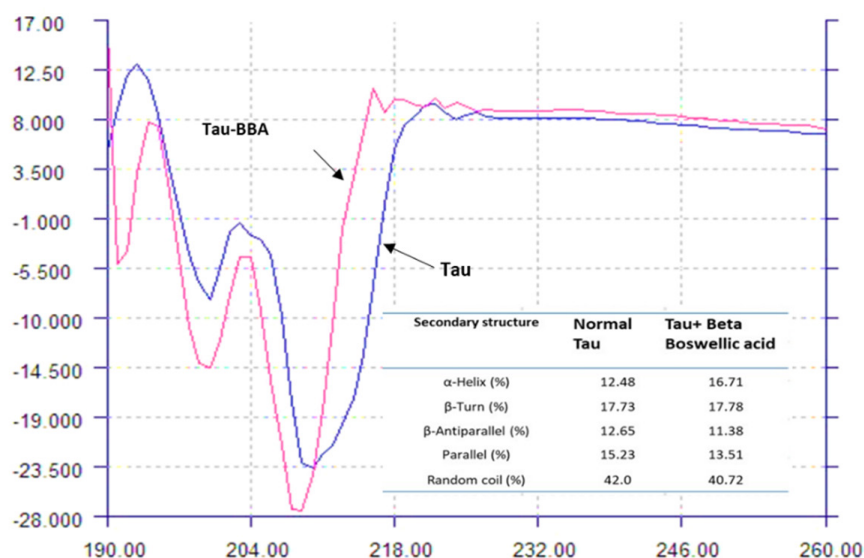


Figure 4. CD spectra of Tau in the absence and presence of BBA (190-260 nm). Tau = 20×10^{-6} M, BBA = 0.5×10^{-6} M.

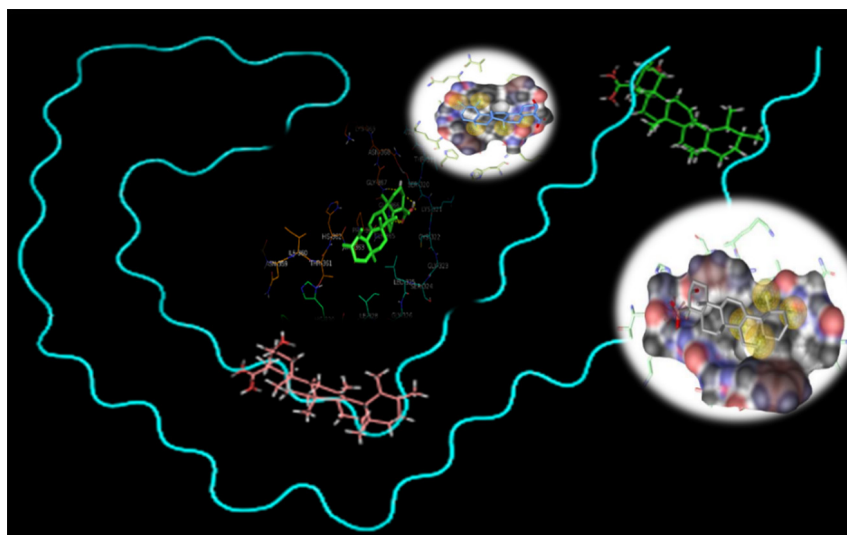


Figure 5. Molecular docking and protein mapping results of Tau–BBA interaction (PDB ID 5OL3).

of ΔH° , and ΔS° and the negative value of ΔG° indicate that the overall thermodynamic stability of Tau-BBA complex comes from an entropy/enthalpy balance. Accordingly, a favorable $T\Delta S^\circ$ term outweighs the unfavorable ΔH° , and the hydrophobic interactions play a major role in the binding of BBA to Tau.

According to the previous studies,²¹ there are different glycation sites on Tau protein; therefore, it is expected to be a common interaction site for BBA and glucose on Tau protein, and the competition between BBA and glucose for interacting with these binding sites can cause the decreased affinity by the increase of glucose concentration. But these underlying mechanisms of the current results are subject to further investigation.

It is well-known that β structures are needed for the aggregation of disordered proteins and studies showed that Tau aggregation is driven by a transition from random coil to β -sheet structure.⁴⁴ while different studies suggested that α -helix could be a driver of primary accumulation and aggregation for disordered proteins.⁴⁵ It seems that BBA induces certain perturbations (increasing α -helical structure) on the Tau protein secondary structure which could affect the physiologic function of Tau.

From the above data, (³⁶²HVPGGG³⁶⁷) segment can be found both in R_2 and R_4 domains. It could be assumed that BBA can interact with both R_2 and R_4 domains, while protein mapping suggested no interaction with a similar segment (i.e. ³³⁰HKPGGG³³⁵) in R_3 . The (PGGG) conserved segments in R_1 - R_4 are responsible for making (PGGG) loops, which have a role in stabilizing microtubules after assembly making.⁴⁶ It is obvious both from SPR studies and molecular docking results that BBA interacts with different repeat domains of Tau, while the crystal structure of Tau is not available.⁴⁷ Therefore, the precise sites of repeat domains of Tau that is involved in the interaction with BBA should be further studied.

Conclusion

We reported the application of SPR and molecular modeling methods for the investigation of BBA interaction with Tau protein. The developed real-time and label-free method was able to determine both kinetic and thermodynamic properties of the interaction readily and accurately.

Results showed that BBA forms a stable complex with Tau ($K_D=8.45\times 10^{-7}$ M) at 298 K. The binding affinity increased by temperature enhancement. The Tau-BBA complex formation decreased significantly in the presence of glucose. It may indicate that there is a common interaction site between BBA and glucose on Tau protein and BBA competes with glucose to interact with these binding sites. Kinetic analysis revealed that both association and dissociation of BBA and Tau are accompanied by an entropic activation barrier; however, the dissociation transition state of the complex is slightly compromised by a favorable enthalpy change. Positive enthalpy and entropy changes revealed that hydrophobic interactions are the main force of interaction between BBA and Tau. These results were in agreement with molecular modeling analysis which showed that BBA can be situated near the (³⁶²HVPGGG³⁶⁷) segment of R_2 and R_4 repeated domains of Tau through hydrophobic interactions.

The probable effect of BBA on Tau aggregation and also interfering of glucose with the binding of BBA to Tau molecule could be further investigated via in vitro studies. Taking into account the potential therapeutic uses of BBA in neurodegenerative disorders such as AD, the developed method would allow researchers to gain a better understanding of the mechanism of action of BBA and also other food-derived compounds in these diseases.

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Ethical statement

The authors declare no ethical issue to be considered.

Competing interests

The authors declare no conflicts of interest.

Authors' contribution

MRR, SRAH, HH: conceptualization and writing & reviewing; MRR, HH: data handling; FF, HH: experiments design; SS, HH: data analysis; SK, FM, HH: provision of study materials and equipment; SS, HH: study validation; MRR, SRAH: supervision; MRR, HH: data presentation, draft preparation and project administration; SK, FM, HH: study consultation;

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Research Highlights

What is the current knowledge?

- ✓ BBA interferes with AD path-ways.
- ✓ BBA binds to the microtubules.
- ✓ Tau protein aggregation is one of the pathological hallmarks of AD.
- ✓ Hyperglycemia relates to Diabetes and AD.

What is new here?

- ✓ Hydrophobic interactions are the main force of BBA-Tau interaction.
- ✓ BBA binds to the Tau protein.
- ✓ BBA situates near the (³⁶²HVPGGG³⁶⁷) segment of R2 and R4 which are important for Tau aggregation.
- ✓ The binding affinity of BBA to Tau decreases significant-ly in the presence of Glucose.

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