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Research Article

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Investigation on the Binding properties of a Coumarin Derivative to Insulin by Spectroscopic and Computational Approaches

Bir Kumarin Türevinin İnsüline Bağlanma Özelliklerinin Spektroskopik ve Hesaplamalı Yaklaşımlarla İncelenmesi

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ABSTRACT

The binding properties of insulin hormone to the potential antidiabetic coumarin derivative umbelliferone (7hydroxycoumarin, 7HC) was investigated by absorption, fluorescence quenching and molecular docking methods. The negative signs of thermodynamic parameters (ΔH and ΔS) indicated that hydrogen bonds and van der Waals forces were dominant in the binding mode. The effect of common metal ions was investigated on binding parameters. According to the Förster's theory; binding distance, r was obtained as 4.17 nm. The spectral data further supported by molecular docking calculations which show hydrogen bonds between 7HC and insulin.

Key Words

Insulin, fluorescence quenching, thermodynamic parameter, FRET.

ÖZ

İnsülin hormonunun potansiyel antidiyabetik kumarin türevi olan umbelliferon (7hidroksikumarin, 7HC) ile bağlanma özellikleri absorpsiyon, floresan söndürme ve moleküler kenetlenme yöntemleriyle incelenmiştir. Termodinamik parametrelerin (ΔH ve ΔS) negatif işaretleri, hidrojen bağlarının ve van der Waals kuvvetlerinin bağlanma modunda baskın olduğunu göstermiştir. Bazı genel metal iyonlarının bağlanma parametreleri üzerindeki etkisi araştırılmıştır. Förster teorisine göre bağ uzunluğu, r, 4.17 nm olarak bulunmuştur. Spektral veriler, 7HC ve insülin arasındaki hidrojen bağlarını gösteren moleküler kenetlenme hesaplamaları ile desteklenmiştir.

Anahtar Kelimeler

İnsülin, floresans sönüm, termodinamik parametre, FRET.

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INTRODUCTION

Diabetes mellitus is an endocrine disorder caused by hyperglycemia, disturbances of carbohydrate, fat and protein metabolism. Hyperglycemia or high blood sugar is a serious problem in diabetes. Therefore, a model antidiabetic agent to be used should have both hypoglycemic and antioxidant properties and also it should not be side effects [1-3]. Umbelliferone (7HC) is one of coumarin derivatives which is known to be effective as an antidiabetic agent in natural products such as edible fruits and vegetables. The significant antihyperglycemic and antioxidant effect of umbelliferone in streptozotocin-diabetic rats were revealed by Ramesh and Pugalendi [4,5]. There are many studies reported about investigation of biophysical effect of umbelliferone on biomolecules [6,7]. The parent molecule coumarin (1,2 benzopyrone) is one of these attractive groups because of the blood glucose level reducing effect its anti-inflammatory, anti-mutagenic, anti-cancer and antioxidant properties. Beside these properties its presence in a wide variety of plants providing high interest to coumarin [8-10].

Insulin is a polypeptide hormone produced by the β-cells of the pancreatic islets and it plays main role in the control of the glucose metabolism and diabetes treatment. The roles of insulin in lowering blood glucose, regulating carbohydrate and fat metabolism make the rapid and sensitive determination of insulin is important [11,12]. An insulin molecule is composed of two chains connected with two disulfide bridges. The chain-A contains 21 amino acid residues and chain-B contains 30 ones. The difference between the insulin of various species of mammals comes from the only by the sequence of amino acids 8, 9 and 10 in the chain-A which has four tyrosine (Tyr) and three phenylalanine (Phe) residues which give fluorescence property to the hormone. In this case Try residues are the major contributors to the absorption spectrum of insulin and emission spectrum because of the low fluorescence quantum yield of phenylalanines [13,14]. Tracking the intrinsic fluorescence of proteins is a common method in the investigation of their physicochemical and structural properties. The aim of the represented study is investigation of the interaction of insulin with 7HC by fluorescence spectroscopy and UV-visible absorption spectroscopy and molecular docking studies. The study is focused on the evaluation of interaction properties, thermodynamic parameters, energy transfer and intermolecular distance of insulin-7HC and the effect of common metal ions on interaction parameters by using fluorescence quenching of intrinsic Tyr emission by 7HC. Here, the results will provide useful contribution to the design and understanding of interaction mechanism of novel coumarin derivatives to biomolecules.

MATERIALS and METHODS

Reagents

Insulin (from bovine pancreas; MW: 5733 Da) and umbelliferone (7hydroxycoumarin) were obtained from Sigma-Aldrich and Fluka, respectively. Insulin stock solution (0.564 mg/mL) was prepared in Tris-HCl buffer solution (0.05 M Tris, 0.1 M NaCl, pH 7.4). 7HC stock solution ($5.0x10^{-3}$ M) was prepared in DMSO. For ionic effect assays, metal ion solutions at $1.0x10^{-3}$ M concentration were prepared in water using their metal salts. Al^{3+}, Co^{2+}, Zn^{2+}, Ca^{2+} and Mn^{2+} ion solutions were prepared from their chlorides and also Pb^{2+} and Cu^{2+} ion solutions were prepared from their nitrates. The daily experimental solutions were prepared by appropriate dilution of stock solutions with buffer. All the solutions were stored in refrigerator 4 ºC until used and doubly distilled water was used in all the carried experiments.

Apparatus

All fluorescence spectra and measurements were performed on a Hitachi F-4500 spectrofluorometer (Japan) which contains FLSolutions software and 150 W Xe lamp. Excitation/emission band slits were 2.5 nm, PMT voltage was 700 V and scan speed was 20 nms^{-1} in all measurements. All absorption measurements were recorded by a Shimadzu UV-1700 PharmaSpec (Japan) UV-visible spectrophotometer which contains UVProbe software. The pH value of solutions was determined with a Mettler Toledo (FiveEasy Plus) digital pH-meter.

Fluorescence measurements and UV-Vis absorption

Fluorimetric titration experiments were carried out manually by using trace syringes. Certain amount of stock umbellifeone solutions were added into 2.5 mL, $2.5x10^{-6}$ M insulin solution in fluorescence cell. The fluorescence spectra were recorded at $\lambda_{ex}/\lambda_{em}$=276/307 nm at three temperatures (296, 303 and 310 K). Stern-Volmer equation was used in the analyses of evaluated fluorescence titration data. According to Förster's resonance energy transfer theory, FRET parameters (r, R_0, $J(\lambda)$, E) were obtained between insulin and 7HC. The following equation was used in the correction of measured tyro-

sine emission data for inner filter effect [15],

$$F_{cor} = F_{obs}\, 10^{(Aex + Aem)/2} \quad (1)$$

Here, F_{cor} and F_{obs} are corrected and observed fluorescence intensities; A_{ex} and A_{em} are absorbances at excitation and emission wavelengths.

The UV-vis absorption spectra were recorded from solution of free 7HC, free insulin and 7HC-insulin mixture in the range of 200-450 nm.

Molecular docking studies

The computational calculations were performed on Fujitsu workstation using Discovery Studio (DS) 2017 R2 [16]. Dock Ligands (CDOCKER) protocol was applied for understanding docking interactions between 7HC and insulin. CDOCKER is a grid based molecular docking technique that uses CHARMm force field [17]. The structure of bovine insulin was downloaded from the Protein Data Bank (PDB) (https://www.rcsb.org/pdb, PDB ID: 4M4L [18], Frankaer, 2014). The ligand, 7HC was drawn and optimized at DFT/B3LYP/6-31G* level by using Gaussian 09 (G09) [19]. After these, DS software was used for preparation of 7HC and insulin to do molecular docking. In the meantime, conformations of 7HC were examined using ligand conformational analysis protocol. CDOCKER was implemented using default settings. The best conformational pose for 7HC against insulin was preferred based on the Binding energy, CDOCKER scores, and root mean square deviation (RMSD) values.

RESULTS and DISCUSSION

Fluorescence quenching studies

Bovine insulin has seven aromatic amino acids, four Tyr (A14, A19, B16, B26) and three Phe (B1, B24, B25) residues, and only three Tyr residues contribute the insulin fluorescence. The disulfide bridge blocks one of the Try residues hence its contribution to the insulin fluorescence. In the case of Phe, fluorescence quantum yield of Try is much higher than the Phe fluorescence quantum yield when it is compared and its contribution to insulin fluorescence can be neglected [20,21]. Fig. 1 shows that 7HC causes a concentration dependent quenching of the intrinsic fluorescence of tyrosine residues of insulin at $\lambda_{ex}/\lambda_{em}$=276/307 nm. The constant amount of insulin in fluorescence cell was titrated with various concentration of 7HC in the range of 3.99-35.4x10^{-6} M. The fluorescence intensity of insulin decreased regularly with the increasing concentration of 7HC without changing shift of emission wavelength and shape of the peaks.

The fluorescence quenching processes are mainly categorized as dynamic or static by means of quenching mechanisms. Quenching mechanism types can be distinguished by temperature dependence of quenching and binding constant values of the investigated systems.

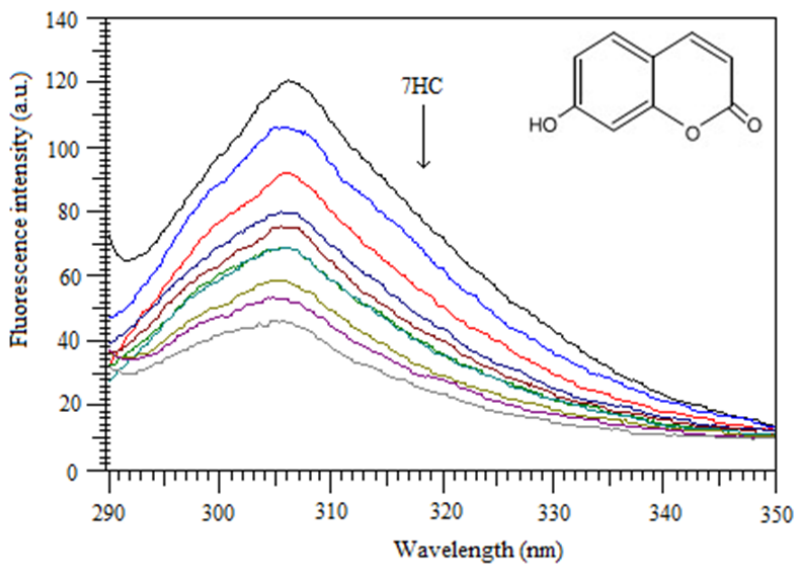

Figure 1. Fluorescence spectra of 5.0x10^{-6} M insulin in the presence of 7HC. The adding concentrations of 7HC (from high to low): 0; 3.99x10^{-6}, 7.97x10^{-6}, 11.9x10^{-6}, 15.9x10^{-6}, 19.8x10^{-6}, 23.7x10^{-6}, 27.6x10^{-6}, 31.5x10^{-6}, 35.4x10^{-6} M. Condition: λ_{ex}= 276 nm, pH 7.4, 296 K. (Inset, molecular structure of 7HC).

Change in excited-state lifetime of the fluorophors is another main parameter in the identification of the interaction mechanisms. Stern-Volmer equation is commonly used in the analyses of fluorescence quenching [15],

$$F_0 / F = 1 + K_{sv}[Q] = 1 + k_q \tau_0 [Q] \quad (2)$$

F_0 is the initial fluorescence intensity of the insulin and F is the fluorescence intensity of insulin in presence of the quencher (7HC). [Q] is the quencher concentration. K_{sv} and k_q represent the Stern–Volmer quenching constant and the quenching rate constant of the biomolecule, respectively. k_q values can be obtained from K_{sv} values by using the average lifetime of the biomolecule without quencher (τ_0:10^{-8} s) [22]. The linear Stern-Volmer plots obtained by using the fluorescence titrations data at different temperatures (296, 303 and 310 K) were shown in Fig. 2a. The values of K_{sv} were obtained from the slopes of graphs. K_{sv} and k_q values represented in Table 1 and the values showed that the quenching constants decreased with the increase in temperature. Accarding to this, the possible fluorescence quenching of insulin by 7HC was static. The reported maximum k_q value of various quencher/biopolymer systems is $2x10^{10}$ $Lmol^{-1}s^{-1}$ [15,23]. The evaluated k_q values of insulin quenching initiated by 7HC were greater than maximum k_q value of dynamic quenching processes. This result proves that the static quenching is driven interaction type with the formation of insulin-7HC complex.

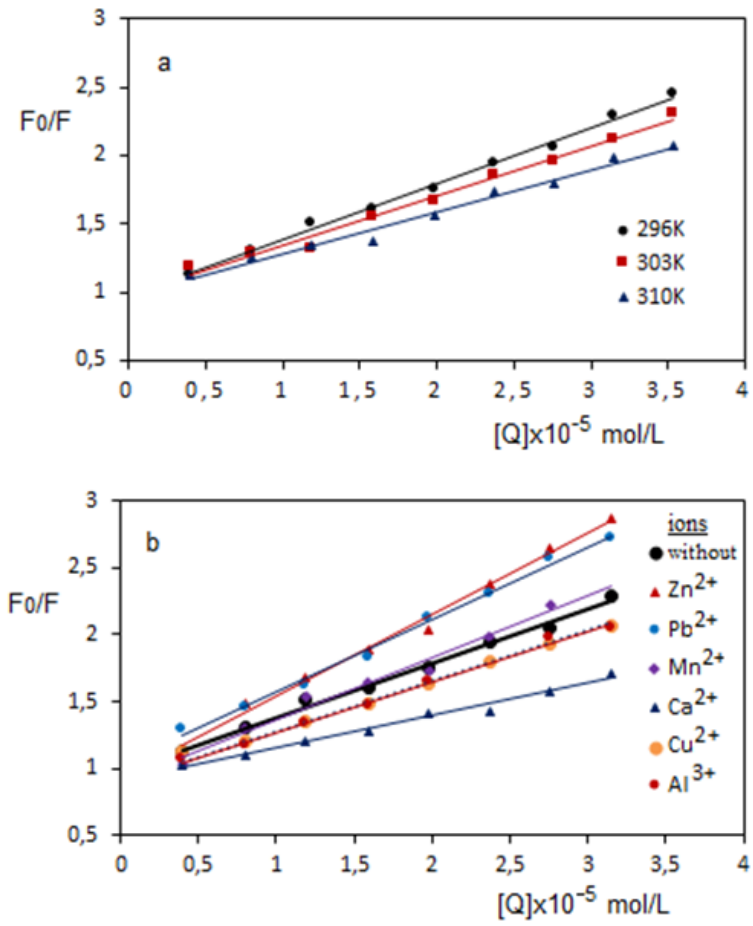

Figure 2. The effect of (a) temperature and (b) common metal ions on the Stern-Volmer plots of 7HC/insulin system. $C_{insulin}$ = 5.0x10^{-6} M, $C_{each\ ion}$ = 10x10^{-6} M at $\lambda_{ex}/\lambda_{em}$ = 276/306 nm.

The binding constant values (K_b) and the binding site numbers (n) of 7HC with insulin were derived from experimental data by using the following double-log equation [24],

$$\log (F_0–F) / F = \log K_b + n \log[Q] \quad (3)$$

Here, K_b and n refer to the binding constant and the binding site numbers of 7HC/insulin system, respectively. As seen in Fig. 3, K_b and n values at three temperatures were obtained from the intercept and slope of the double-log plot, log (F_0–F)/F versus log [*Q*], respectively. The K_b and n data were listed in Table 1. The decrease of the binding constants with increasing temperature indicates that the complex structure is partially decomposed at high temperature.

Common metal ion effects on the Stern-Volmer quenching and binding constants

Binding properties of plasma proteins with small molecules can be altered by metal ions [25, 26]. The effect of several common metal ions was investigated on the interaction of 7HC/insulin. The changes in the quenching constant, binding constant and the number of binding sites of 7HC/insulin complex were examined in the presence of Zn^{2+}, Pb^{2+} Al^{3+}, Ca^{2+}, Cu^{2+}, Mn^{2+} and Co^{2+} ions at 296 K. All fluorescence spectra of 7HC/insulin system were recorded in the range of 290-350 nm at excitation 276 nm. The final concentrations of insulin and metal ions were fixed $5.0x10^{-6}$ M and $10x10^{-6}$ M in titrations, respectively. The effect of these metal ions on Stern-Volmer plots were shown in Fig. 2b. The quenching constants, binding constants, the ratio of the quenching constants or binding constant in the presence of metal ion (K_{SV}' or K_b') to the quenching constants or binding constants in the absence of metal ions (K_{SV} or K_b) and binding site numbers were calculated and represented in Table 2. The higher K_{SV}' values were obtained in the presence of Zn^{2+}, Pb^{2+} and Mn^{2+} ions which point that the quenching effect becomes more significant when both ion and 7HC are present together. The K_{SV}' values are smaller in the presence of Ca^{2+} and Cu^{2+} ions which can be resulted inhibition of quenching effect of 7HC by ion-7HC interaction.

The higher binding constants and also n values in the presence of Al^{3+}, Ca^{2+} and Cu^{2+} ions can be caused from ion-7HC interaction via metal ion bridge. This may pro-

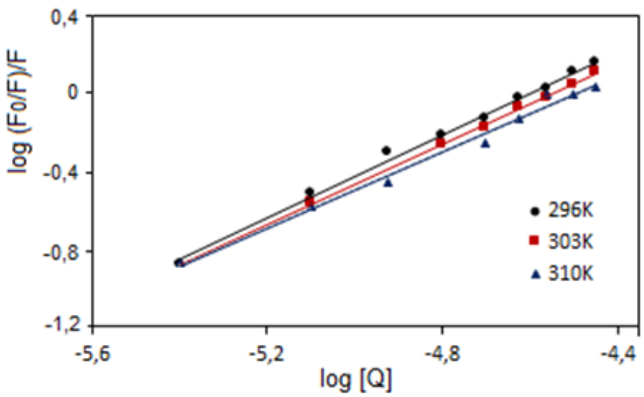

Figure 3. The double-log plots for the binding of 7HC with insulin at different temperatures.

Table 1. Stern-Volmer quenching (Ksv), quenching rate (kq), binding (Kb) constants and binding sites (n) for 7HC/insulin system at different temperatures.

T (K)	Ksv (M^{-1})x10^4	kq($M^{-1}s^{-1}$) x10^{12}	R^{2*}	Kb (M^{-1})x10^4	n	R^{2**}
296	4.108	4.108	0.995	7.849	1.064	0.994
303	3.616	3.616	0.987	4.927	1.032	0.997
310	3.048	3.048	0.981	2.460	0.978	0.990

*the correlation coefficient for the Stern-Volmer plot.
**the correlation coefficient for the Kb value from double-log plot.

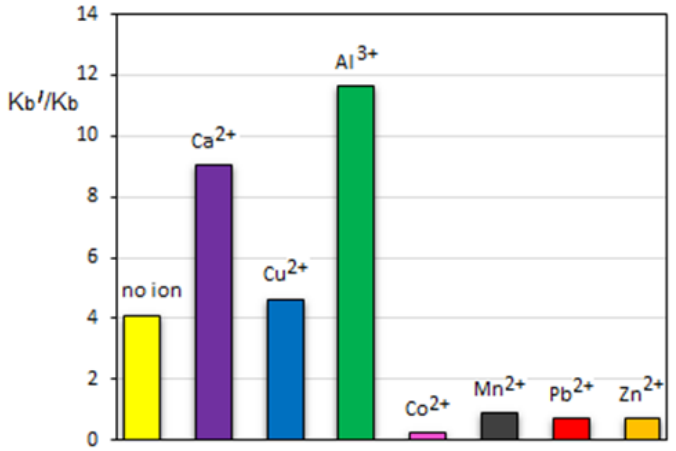

Figure 4. The ratio of binding constants versus metal ions for 7HC-insulin system.

Table 2. Common metal ions effect on the Stern-Volmer quenching (K_{sv}) and binding (K_b) constants and binding sites (n) numbers of 7HC/insulin system at 296 K

Ions	K_{sv}ı ($Lmol^{-1}$) x10^4	K_{SV}'/K_{SV}	R^{2*}	K_b' ($Lmol^{-1}$) x10^4	K_b'/K_b	n	R^{2*}
without	4.108	--	0.9947	7.849	---	1.064	0.9944
Ca^{2+}	2.432	0.592	0.9829	71.12	9.061	1.336	0.9868
Cu^{2+}	3.523	0.856	0.9953	36.42	4.640	1.226	0.9976
Al^{3+}	4.038	0.983	0.9876	91.47	11.65	1.314	0.9884
Co^{2+}	4.098	0.998	0.9816	1.731	0.221	0.9075	0.9976
Mn^{2+}	4.673	1.138	0.9798	6.947	0.885	1.048	0.9906
Pb^{2+}	5.414	1.318	0.9933	5.493	0.700	0.9997	0.9925
Zn^{2+}	6.150	1.497	0.9919	5.645	0.719	0.9983	0.9899

K_b' and K_{sv}' represent the binding and quenching constants in the presence of metal ions, respectively. *regression coefficients of plots.

long the storage period of 7HC in plasma and enhance its maximum effects or ions induced the conformational changes of insulin which is easier for 7HC to insulin. Overhand the decrease in the binding constant in the presence of Co^{2+}, Zn^{2+}, Pb^{2+} and Mn^{2+} ions may due to the formation of metal ion-insulin ionic interaction which is likely to affect the conformation of insulin. It may influence 7HC binding kinetics and imply weaker binding between 7HC and insulin. Binding site numbers represented in Table 2 show that the n values in the presence of these ions are smaller than n value obtained in the absence of metal ions, 1.064. It can be concluded that the 7HC can be removed from 7HC/insulin complex because of the competition between 7HC and ions. The decrease in binding constant could point that the shortening in the storage time and enhancement in the effectiveness of 7HC in plasma. The ratio of binding constants (K_b'/K_b) versus metal ions is shown in Fig. 4.

Thermodynamic analysis and binding mode

Thermodynamic analysis of the binding parameters of small molecules is one of the common ways to characterize the acting forces. Noncovalent interactions between small molecules and biomolecules mainly categorized in four groups; hydrogen bonds, van der Waals forces, electrostatic forces and hydrophobic interactions [27]. The main acting force in the interaction determine the signs and magnitude of the enthalpy (ΔH) and entropy change (ΔS) of the system. The thermodynamic parameters of the interaction of 7HC with insulin were analyzed by using van't Hoff equation:

$$\ln K_b = - \Delta H/RT + \Delta S/R \quad (4)$$

The obtained ln K_b values from the fluorimetric titrations at different temperatures were plotted against the reciprocal of studied temperatures. According to the

van't Hoff graph (Fig. 5), ΔH and ΔS values were obtained from the slope and intercept of linear plot, respectively. The free energy change (ΔG) of the system at different temperatures can be obtained with the following relationship:

$$\Delta G = \Delta H - T\Delta S = -RT \ln K_b \quad (5)$$

The parameters are calculated and listed in Table 3. The negative sign of ΔG indicates the spontaneous binding process of 7HC-insulin system. The negative enthalpy change of the complex points that the mainly enthalpy driven exothermic binding process. According to the theory of Ross and Subramanian about the characteristic signs of the thermodynamic parameters in protein association process [28], the negative ΔH and ΔS values confirm that the hydrogen bonding or van der Waals forces in binding mechanism of molecules to proteins, which is consisted with the values obtained from the interaction of 7HC with insulin [29].

Ultraviolet-visible absorption studies

Fig. 6 shows the absorption spectra of free insulin, free 7HC and 7HC-insulin system at 296 K. The absorption intensity of the insulin absorption band observed at 278 nm increased by the addition of 7HC. Beside that the absorption band of 7HC with the absorption maxima at 325 nm was disappeared in the presence of insulin. This result indicates that the reason of the change in absorption spectra is formation of a ground state complex between 7HC and insulin.

Energy transfer from insulin to 7HC

Fluorescence energy transfer (FRET) is a process the transfer of excited state energy from a donor to an acceptor. It takes place simultaneous quenching of the donor fluorescence and electronic excitation of the acceptor in the process [30,32]. According to Förster's non-radiative energy transfer theory, the energy transfer depends on the overlap of fluorescence spectrum of the donor with UV-vis absorption spectrum of the acceptor and the distance of approach between donor and acceptor. The energy transfer efficiency E can be calculated under the condition of 1:1 situation of donor and acceptor concentrations by using the Eq. 6;

$$E = 1 - F / F_0 \quad (6)$$

$$E = R_0^6 / (R_0^6 + r^6) \quad (7)$$

Here, F and F_0 are fluorescence intensities of insulin with and without 7HC; r is acceptor-donor distance; R_0, critical distance refers to the distance 50% transfer efficiency provided. The energy transfer efficiency is changed with acceptor concentration at different temperatures.

R_0 value is calculated by using the following equation;

$$R_0 = 0.211 \left(\kappa^2 n^{-4} Q_D J(\lambda) \right)^{1/6} \quad (8)$$

κ^2: The spatial orientation factor of the donor-acceptor dipoles

n: Working medium refractive index

Q_D: Initial fluorescence quantum yield of the donor

$J(\lambda)$ is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor and it can be calculated by the Equation 9;

$$J(\lambda) = \sum F_D(\lambda)\, \varepsilon_A(\lambda)\, \lambda^4\, \Delta\lambda \,/\, \sum F_D(\lambda)\, \Delta\lambda \quad (9)$$

$F_D(\lambda)$ is the corrected fluorescence intensity of the donor in the wavelength range λ to $\lambda+\Delta\lambda$ with the total

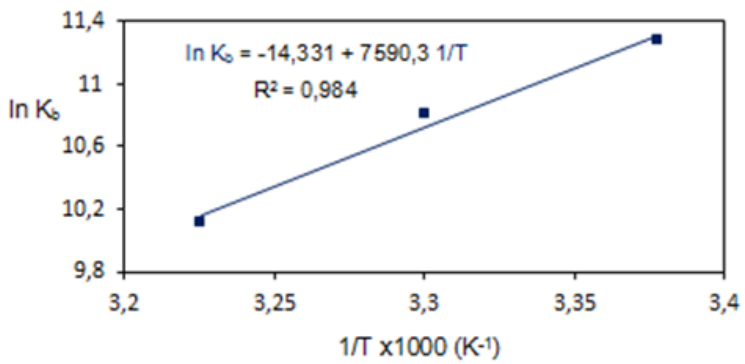

Figure 5. The van't Hoff graph of 7HC-insulin system.

Table 3. Thermodynamic parameters of 7HC/insulin system.

T (K)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (kJ/mol)	Binding mode
296	-63.11	-27.84	-119.2	Hydrogen bonding van der Waals forces
303		-27.00		
310		-26.17		

intensity (area under curve). $\varepsilon_A(\lambda)$ is the extinction coefficient of the acceptor at λ. $J(\lambda)$ value could be evaluated by integrating the overlap of absorption spectrum of 7HC (acceptor) and fluorescence spectrum of insulin (donor) in Fig. 7. The integration result for λ = 290-350 nm wavelength range was 3.8807×10^{14} $Lmol^{-1}cm^3nm^4$ according to Eq. 9. The obtained value of E by using Eq. 6 was 0.1598 at the equal concentrations of insulin and 7HC. r and R_0 were calculated as 4.17 and 3.16 nm by using Eq. 7, respectively. In the calculation required values used as $\kappa^2 = 2/3$, $n = 1.336$ and $Q_D = 0.14$ for tyrosine [15]. The results showed that the donor-acceptor distance (r) is less than 8 nm, the value is in the range of $0.5R_0 < r < 1.5\ R_0$, which suggests the non-radiative energy transfer from insulin to 7HC. Also, $r > R_0$ support the presence of a static quenching [33].

Analytical results and determination of 7HC

The regular quenching of the intrinsic tyrosine emission of insulin with increase in 7HC concentration was observed from fluorimetric titration which was carried out at fixed insulin concentration by adding 7HC solutions. The linear Stern-Volmer graph ($F_o/F = 0.9688 + 4.108 \times 10^4$ [Q] with R=0.9947) was used determination of 7HC in the presence of insulin at 296 K. 3*Sb/m* and 10*Sb/m* were used to obtain the LOD (limit of detection) and LOQ (limit of quantification) values of 7HC, respectively. *m* is the slope of the calibration graph and *Sb* is the standard deviation of the intercept. *N* is the number of replicate measurements [34]. The obtained results (N=7) were listed in Table 4. The relative standard deviation (RSD) was obtained as 2.93% from replication of quenching results of 7.97×10^{-6} M concentration of 7HC (N=8) for the precision of the method.

Molecular Docking Studies

In this section, molecular docking method is employed to predict the structure of the intermolecular complex formed between 7HC and insulin. The ligand, 7HC was docked with insulin and the best docked pose in all possible ten poses of 7HC is shown in Fig. 8. In the docked complex, the 7HC has two hydrogen bonds acceptor interactions with the hydroxyl oxygen atom O1 of TYR19 of active site of insulin at distances 1.795 Å. and also with GLY1 residue of active site of insulin at distance 2.447 Å. The docked 7HC-insulin complex and its docked interactions are shown in Fig. 8 and Table 5, respectively.

It is evident that the complex is stabilized mostly due to hydrogen bonds and van der Walls interactions. Doc-

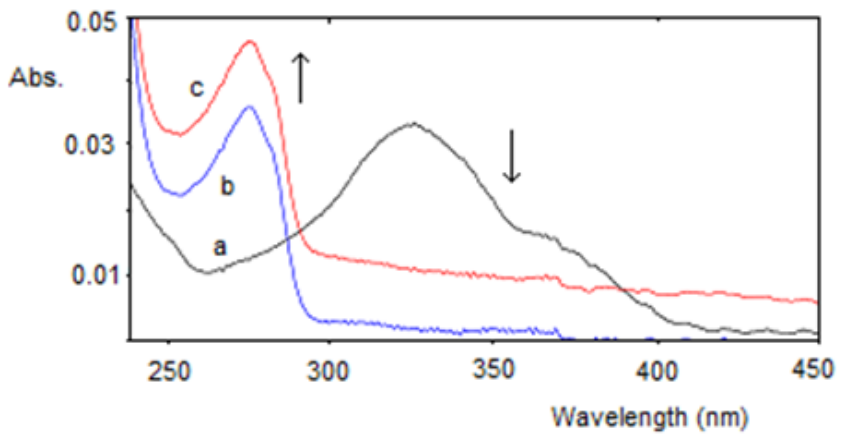

Figure 6. The absorption spectra of (a) 5.0×10^{-6} M free 7HC, (b) 5.0×10^{-6} M free insulin, (c) 5.0×10^{-6} 7HC-5.0×10^{-6} insulin system.

king score, docking interaction, binding and entropic energies and solvent accessible surface area buried and root mean square deviation values of 7HC compound with insulin are exhibited at the end of the molecular docking. The obtained binding energy and solvent accessible surface area (SASA) values (-12.845; 47.88, respectively) of ligand show the affinity of ligand and how well bonding was done with insulin as given in Table 6.

The docking results reveal that 7HC compound was held in the binding site by various hydrogen bonds and van der Waals interactions with bovine insulin. These interactions are very important in enhancing binding affinity and the biological activity of the compound. Additionally, the lowest CDOCKER, CDOCKER interaction energy values and negative binding and entropic energies support better docking complex of the 7HC. These results may be help for related studies to evaluate its advanced clinical studies.

CONCLUSION

The interaction of insulin hormone with umbelliferone (7Hydroxycoumarin, 7HC) which is a coumarin derivative was investigated with spectral and molecular docking studies. Obtained results showed that 7HC has strong ability to quench the tyrosine fluorescence of insulin by static quenching process with formation a ground state complex. Binding constants and binding site numbers and the effect of common metal ions on these values were explored from the fluorescence data. In the presence of Al^{3+}, Ca^{2+} and Cu^{2+} ions increase in binding constants and n values were observed. One can be concluded that these ions enhance the storage period of 7HC in plasma and its maximum effects. On the other hand the decrease in the binding constant in the presence of Co^{2+}, Zn^{2+}, Pb^{2+} and Mn^{2+} ions can be used to easy decomposition of 7HC-insulin complex by competitive binding of metal ions to insulin. The energy transfer calculations performed based on the Förster theory and r value was obtained as 4.17 nm which indicates the non-radiative energy transfer from insulin to 7HC. For determination of 7HC in the presence of insulin, LOD and LOQ were calculated as $2.95x10^{-6}$ M and $9.83x10^{-6}$ M 7HC in this method, respectively. The obtained negative ΔH^{o} (-63.11 $kJmol^{-1}$) and ΔS^{o} (-119.2 $Jmol^{-1}K^{-1}$) values mark that the hydrogen bonding and van der Waals forces are dominant forces in stabilizing the complex. The binding reaction was spontaneous and exothermic process. According to molecular docking calculations, the complex includes two hydrogen bonds with GLY1 and TYR19 residues of insulin. Binding energy, solvent accessible surface area and root mean square deviation were also obtained to be -12.845 $kcalmol^{-1}$, 47.88% and 0.00968, respectively. The binding of 7HC to insulin may be provided by increased secretion of insulin and more decreased blood glucose levels. Furthermore, the ob-

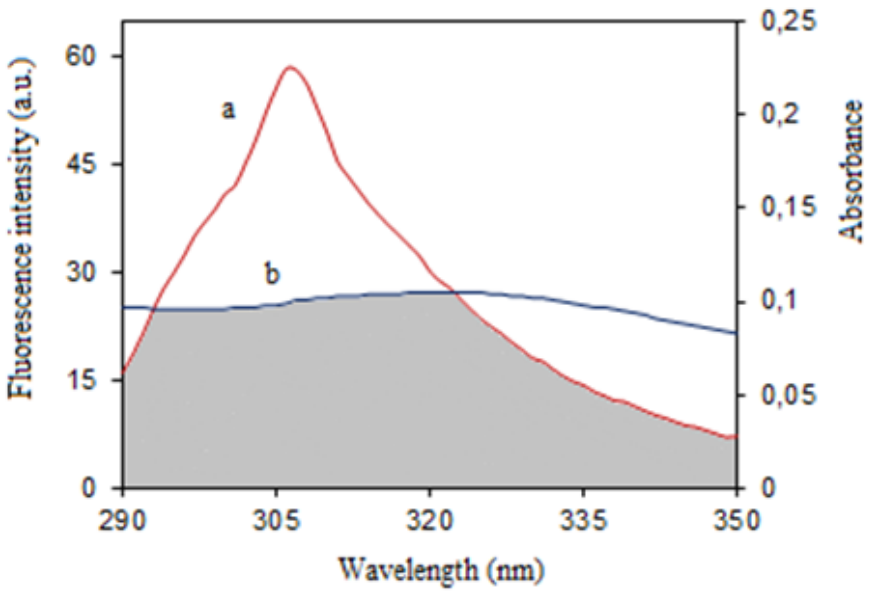

Figure 6. The absorption spectra of (a) $5.0x10^{-6}$ M free 7HC, (b) $5.0x10^{-6}$ M free insulin, (c) $5.0x10^{-6}$ 7HC-$5.0x10^{-6}$ insulin system.

Table 4. The analytical results for determination of 7HC in the presence insulin.

Dynamic range of 7HC (M)	$3.99 \times 10^{-6} - 35.4 \times 10^{-6}$
Standard deviation of the intercept (*Sb*)	0.04037
Limit of detection (LOD) (M)	2.95×10^{-6}
Limit of quantification (LOQ) (M)	9.83×10^{-6}

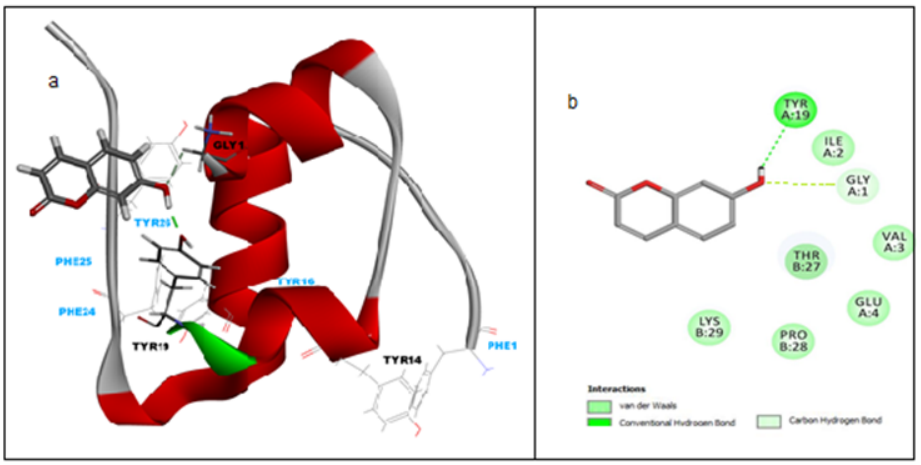

Figure 8. Docked 3- and 2-dimensional interactions of 7HC compound to insulin.

tained experimental results have been confirmed with molecular docking results which show that the compound may act as an antidiabetic agent against insulin. In addition, this study can be a guide to evaluate the interaction behavior of a coumarin derivative and insulin with experimental and computational applications.

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Table 5. Interaction data between 7HC and insulin.

Compound	Bonding	Bonding Types	Distance (Å)	Interactions
7HC	H Bond	Conventional H Bond	1.795	7HC:H18 - A:TYR19:OH
	H Bond	Carbon H Bond	2.447	A:GLY1:HA1 – 7HC:O12

Table 6. Molecular docking score and energies of 7HC.

Compound	Docking Score Energy	Docking Score Interaction Energy	Binding Energy (kcalmol-1)	Entropic Energy (kcalmol-1K-1)	Solvent Accessible Surface Area Buried %	Root Mean Square Deviation
7HC	-9.687	-12.734	-12.845	-26.657	47.88	0.00968

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