Hacettepe University Journal of the Faculty of Pharmacy
Volume 32 / Number 1 / January 2012 / pp. 1-16

Investigation of Interaction of Simvastatin with Human Genomic DNA

Received : 14.12.2011
Revised : 19.02.2012
Accepted : 20.03.2012

Mehmet Lütfi Yola*º, Nuran Özaltın*

Introduction

Simvastatin (SIM) (Figure 1) reduces levels of circulating atherogenic lipoproteins by competitive inhibition of the microsomal enzyme 3-hydroxy-3-methylglutaryl-co-enzyme A(HMG-CoA) reductase, which catalyzes the conversion of HMG-CoA to mevalonate, a critical intermediary in the biosynthesis pathway of cholesterol[1]. Also SIM inhibits oxidation of native and modified low-density and high-density lipoproteins[2]. So, it is used in the treatment of hypercholesterolemia[3,4].

Deoxyribonucleic acid (DNA) plays a central role in life process since it contains all of the genetic information required for cellular function. However, DNA molecules are prone to be damaged under various conditions, especially by interaction with some molecules and this damage may lead to various pathological changes in living organisms. Studies on the binding interactions of small molecules with DNA are of interest for both therapeutic and construction of new and more efficient drugs targeted to DNA as well as in understanding how proteins recognize and bind to specific DNA sequences[5-7].

* Hacettepe University, Faculty of Pharmacy, Department of Analytical Chemistry, Ankara, Turkey
º Corresponding author: E-mail: mehmetl@hacettepe.edu.tr

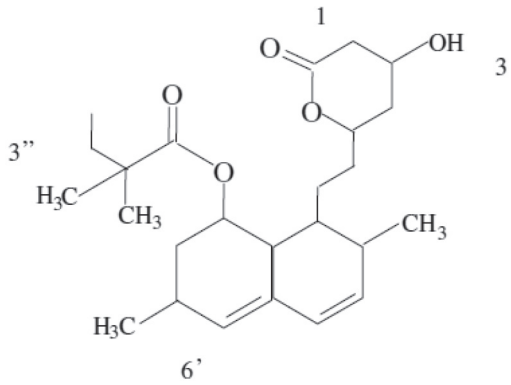


Figure 1
Chemical structure of SIM

A variety of small molecules interact reversibly with double-stranded DNA, primarily through three modes: electrostatic interactions, with the negative charged nucleic sugar-phosphate structure, which are along the external DNA double helix and do not possess selectivity; binding interactions with two grooves of DNA double helix; and intercalation between the stacked base pairs of native DNA[8].

In recent years, electrochemical methods have gained growing interests in the investigation of DNA – drug interaction[9-13]. In this study, detailed investigations on the electrochemical behavior of the interaction between SIM and DNA were carried out using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) at hanging mercury dropping electrode (HMDE). Moreover, UV–vis spectra were studied to characterize the interaction mode and the interaction mechanism. It is suggestive for further fruitful research to quantify the therapeutic efficacy of SIM.

Material and Methods

Genomic DNA isolation from whole blood

Human genomic DNA was isolated from whole blood using ammonium sulfate precipitation method[14]. To confirm the purity of the genomic DNA extract we used A_{260}/A_{280} ratio and solutions of the genomic DNA gave rations of UV absorbance at 260 and 280 nm (A_{260} / A_{280}) of approxi-

mately 2.1, indicating that the genomic DNA was sufficiently protein-free[15]. The purified genomic DNA was run on 1.2 % agarose gel at 10 V/cm for 40 minutes. The gel was stained with Ethidium Bromide and the gel image was acquired using Kodak Molecular Imaging System.

Human genomic DNA (400 μg mL^{-1}) was dissolved in water and stored at 4 ^{0}C. Solutions were incubated at 37 ^{0}C for 2 hours. SIM stock solution (1.0 x 10^{-4} M) were prepared in methanol and kept away from light to avoid photochemical decomposition. Working standard solutions were prepared daily by appropriate dilution of the stock standard solutions. A series of supporting electrolytes [borate, acetate, phosphate and Britton-Robinson] were tested in the presence of 1.64 μg mL^{-1}. The results showed that SIM in Britton-Robinson buffer gave the highest signal response. If not specially stated, the supporting electrolyte was Britton-Robinson (BR) buffer. BR buffers (pH 7.1), ionic strength 0.2 mol dm^{-3}, were prepared (0.04 M of each of acetic, o-phosphoric and boric acids, adjusted to the required pH with 0.2 M sodium hydroxide) and used as supporting electrolytes. All reagents were of analytical grade quality. Deionized water was used throughout to prepare solutions. Proteinase K, agarose, ethidium bromide, FX 174 DNA marker and cyan-orange DNA stain were purchased from Invitrogen (UK). Ammonium Sulfate, SDS, Ethanol, Tris were purchased from Sigma Aldrich (MO,USA).

Instrumentation

BAS 100 B/W model electrochemical workstation was used. The reference electrode was Ag/AgCl and a platinum wire was used as the auxiliary electrode and HMDE was used as the working electrode. Shimadzu Spectrophotometer UV-1800 was used for spectrophotometric analysis. All the pH measurements were made with Metler Toledo MA 235.

Electroanalytical procedure

Prior to the experiments, high purity nitrogen was used to deaeration of 900 μL supporting electrolyte for at least 10 min. Then 100 μL of 1.0 x 10^{-4} M stock solution of SIM was placed into the cell to make up 1000

μL mixture solution at a SIM concentration of 1.0 x 10^{-5} M and N_2 was passed through in 2 seconds. The voltammograms were recorded with cyclic potential scan between -1.0 V and -1.6 V. During the experiments, nitrogen atmosphere was maintained over the solutions to prevent the reentry of atmospheric oxygen. All experiments were typically carried out at room temperature.

Results and Discussion

Interaction of SIM with DNA

The electrochemical behaviour of SIM at HMDE was investigated employing CV and DPV. To prevent DNA from acidic or basic denaturing, BR buffer of pH 7.1 was chosen as supporting electrolyte. The CV behaviour of SIM showed one reduction peak at -1.404 V in BR buffer of pH 7.1 with a scan rate of 100 mV s^{-1} (Figure 2). No peak was observed in the reverse scan suggesting that reduction of SIM at HMDE is irreversible.

The effect of scan rate (v) on the peak current (I_p) of SIM have been studied. For this, we recorded CV of 1.0 x 10^{-5} M SIM at HMDE in the scan rate range of 25 – 300 mV s^{-1} (Figure 3-A). The plots of log I_p versus logv in the scan rate range of 25 – 300 mV s^{-1} yielded a straight line with

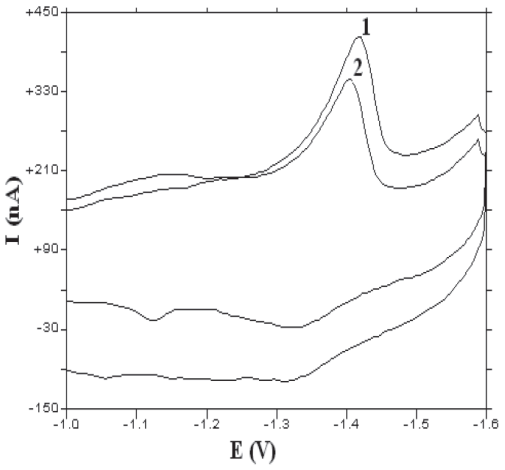


Figure 2
Cyclic voltammograms of 1.0 x 10-5 molL^{-1} SIM in the (1) absence and (2) presence of 1.73 μg mL^{-1} DNA. Supporting electrolyte: Britton–Robinson buffer, pH 7.1, scan rate 100 mVs^{-1}

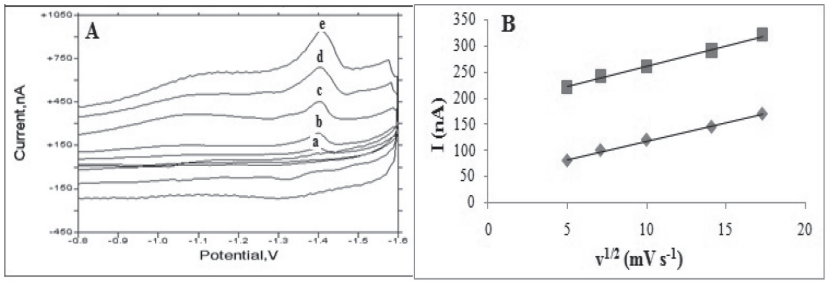


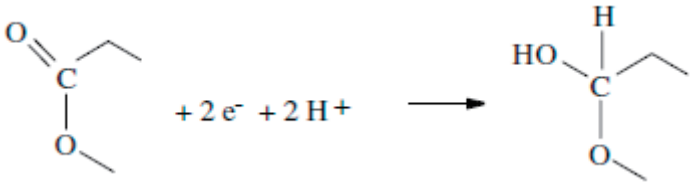
Figure 3

(A) Cyclic voltammogram of 1.0 x 10-5 molL^{-1} SIM at HMDE Supporting electrolyte: Britton–Robinson buffer (pH 7.1) ; (a) 25 mV s^{-1} ; (b) 50 mV s-1; (c) 100 mV s-1 ; (d) 200 mV s^{-1}; and (e) 300 mV s^{-1}

(B) Relationship between the peak currents of the reduction wave of SIM in the absence (■) and presence of (◆) 2.15 μg mL-1 DNA and the square root of scan rates

slope of 0.96 for SIM. These values are close to the therotical value of 1.00, which is expected for an ideal reaction condition for adsorption – controlled electrode process [16,17]. Furthermore, the smaller linear slopes of DNA complex demonstrated (Figure 3-B) that SIM could interact with DNA in solution, forming SIM – DNA adducts with large molecular weight, resulting in a considerable decrease in the apparent diffusion coefficient. Bard and co-workers [18] reported that positive shifts in the peak potential of intercalators were observed in the binding form via hydrophobic interactions (intercalation) while electrostatic interactions led to negative shifts. Based on this report, the positive shifts in the peak potential of SIM upon binding to DNA should be as a result of intercalative interaction to DNA.

The single reduction peak of SIM is attributed to two electron reduction of ester group on the position 1 (Figure 1)[19]. So a possible mechanism is proposed to explain the electrochemical reduction of SIM as below:



The electron transfer coefficient 'α' is calculated from the difference between peak potential (E_p) and half wave potential ($E_{p/2}$) according to

equation given below [20]. The value of electron transfer coefficient is 0.5 for irreversible electrochemical reaction whereas the value of electron transfer coefficient is 1.0 for reversible electrochemical reaction.

$$\Delta E_p = E_p - E_{p/2} = (47.7 / \alpha n)\ mV \text{ (irreversible reaction, at 298K)} \quad (1)$$

The value of α is calculated to be 0.51 ± 0.05 for SIM. So it is suggesting that reduction of SIM at HMDE is irreversible[19]. For an irreversible cathodic reaction, we may use the following equation to calculate standard rate constant [21,22].

$$E_p = E^0 + (RT / \alpha nF)[\ln(RTk_0 / \alpha nF)] - \ln v] \quad (2)$$

Where E^0 is the formal potential, R was the universal gas constant (8.314 J K^{-1} mol^{-1}), T (K) was the Kelvin temperature, α was the transfer coefficient, k_0 (s^{-1}) was the electrochemical rate constant and F was the Faraday constant (96487 C mol^{-1}). The value of E^0 was obtained from the intercept of the E_p versus v plot by the extrapolation to the vertical axis at v = 0. The values of k_0 were evaluated from the plot of E_p versus lnv and found to be 2.31 (± 0.15) x 10^5 s^{-1} for SIM. The value of obtained k_0 was high so it was suggesting that no peak was obtained on the reverse scan.

Furthermore, chronocoulometry was performed in the solutions of 5.0 x 10^{-5} mol cm^{-3} SIM in Britton–Robinson buffer of pH 7.1 as the electrolyte. The diffusion coefficient (D) can be determined according to the formula given by Anson[23]:

$$Q(C) = 2nFACD^{1/2}t^{1/2}\pi^{-1/2} + Q_{dl} + Q_{ads} \quad (3)$$

where A (cm^2) was the surface area of the working electrode, C (mol cm^{-3}) was the concentration of SIM, D (cm^2 s^{-1}) was the diffusion coefficient of SIM, t (s) was time, Q_{dl} (C) was double-layer charge and Q_{ads} (C) was the faradaic charge due to the reduction of adsorbed drug. In our experiment, the effects of double-layer charge Q_{dl} can be eliminated via subtraction of the background charge and the plots Q (μC) against t (s) were converted into the plots of Q against $t^{1/2}$, which was depicted in Figure 4. It was clear that the charges (Q) have linear relationships with the square roots of time ($t^{1/2}$) for the reduction reaction. According to Eq.(3), the diffusion coefficient of SIM and SIM-DNA complex can be estimated from the slope of the plot of Q-$t^{1/2}$. The diffusion coefficients of SIM and SIM-DNA complex were calculated as 8.07 (± 0.25) x 10^{-8} cm^2 s^{-1}, 1.32 (± 0.25) x 10^{-9} cm^2 s^{-1}, respectively. Diffusion coefficients decrease because large complexes decrease mass transfer at electrode surface.

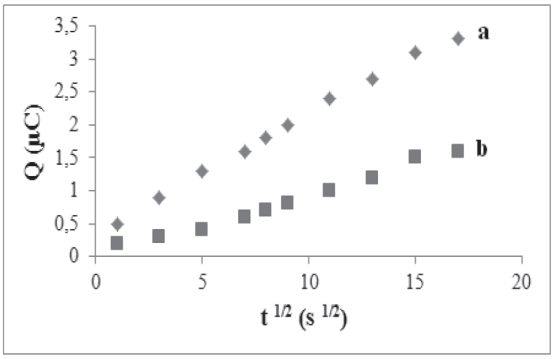


Figure 4
The linear relationship between the charges (Q) and the square-root of time (t1/2) for (a) SIM and (b) for SIM – DNA.

The decrease in peak current and diffusion coefficient of SIM upon addition of DNA may be attributed to several possible reasons. These are as follows: The major electrochemical kinetic parameters (α and k_0) of SIM in presence and absence of DNA can demonstrate whether DNA influences the electrochemical kinetics of SIM or not. The calculated values of α and k_0 are noticed to be 0.51 (± 0.05) and 2.31 (± 0.15) x 10^5 s^{-1} for SIM in absence of DNA and 0.53 (± 0.05) and 2.48 (± 0.20) x 10^5 s^{-1} for SIM – DNA in presence of DNA. In this way, appreciable difference in the values of α and k_0 in presence and absence of DNA was not observed indicating that the DNA did not alter the electrochemical kinetics of SIM reduction. In order to prove that the decrease in peak current is not due to increase viscosity of the solution or the blockage of the electrode surface by DNA adsorption [24]. The special CV experiment was designed in $K_4[Fe(CN)_6]$ solution in presence and absence of DNA at glassy carbon electrode by Kalanur *et al* [25]. The $Fe(CN)_6^{-3/-4}$ ions did not interact with DNA due to coulombic repulsion between their negative charges. The peak currents of $Fe(CN)_6^{-3/-4}$ are observed to be almost same in presence and absence of DNA. The CV experiment was designed in $Fe(CN)_6^{-3/-4}$ solution in presence and absence of DNA at HMDE by us. The voltammograms of $Fe(CN)_6^{-3/-4}$ in presence of DNA are the same as that in absence of DNA, So any interaction was not observed at HMDE. In addition we think that the same behaviour should be valid for the other electrodes in terms of interaction only. Hence, we conclude that the decrease in peak current is not due to change in viscosity of the solution or blockage of the electrode

surface by DNA adsorption. Therefore, the decrease in peak currents of SIM upon the addition of DNA is attributed to decrease in equilibrium concentration of free SIM. Hence, we propose that SIM and DNA formed an electrochemically inactive SIM–DNA complex. Ester group of SIM on the position 1 interacted with DNA so inactive SIM-DNA complex occured with increasing concentration of DNA. This indicates interaction between DNA and drug.

If it is assumed that the interaction of DNA with drugs produces only a single complex, DNA – mDRUG, then the equation shown below holds good[26]:

$$DNA + mSIM \leftrightarrow DNA - mSIM$$

where m is the binding ratio. The equilibrium constant, β_S can be deduced from the following equation:

$$\beta_S=[DNA–mSIM]/\{[DNA][mSIM]^m\} \quad (4)$$

According to the Ilkovic equation for an irreversible electrode process,

$$\Delta I_{max}=kC_{DNA} \quad (5)$$

$$\Delta I=k[DNA—mSIM] \quad (6)$$

$$[DNA]+ [DNA — mSIM] = C_{DNA} \quad (7)$$

$$\Delta I_{max}-\Delta I=k(C_{DNA}–[DNA—mSIM]] \quad (8)$$

$$\Delta I_{max}-\Delta I=k[DNA] \quad (9)$$

From Eqs. (4), (5), and (8) we get

$$(1/\Delta I)=(1/\Delta I_{max})+\{[1/(\beta_S\Delta I_{max})]x(1/[SIM^m])\} \quad (10)$$

or

$$\log[\Delta I/(\Delta I_{max}–\Delta I)]=\log\beta_S+m\log[SIM] \quad (11)$$

where ΔI is the difference in peak current in presence and absence of DNA and ΔI_{max} corresponds to the obtained value when the concentration of SIM is extremely higher than that of DNA. C_{DNA}, [DNA] and [DNA – mSIM] are the total, free and bound concentrations of DNA in the solution, respectively. If DNA interacts with SIM to form a single complex, then the plot of log [ΔI /(ΔI_{max} - ΔI)] versus log [SIM] shows linearity. The values of binding ratio and binding constant are obtained from the slope and intercept, respectively, and these values are found to be 2.0 and

1.66 (± 0.10) x 10^4 M^{-1} for SIM-DNA. Thus, the formation of a stable 1:2 complex (DNA:SIM) are proposed.The other method for the calculation of binding constant is Bard's method:

Amperometric current titrations were performed by keeping the constant concentration of the drug while varying the concentration of DNA using both DPV and CV at pH 7.1. The interaction of drug with DNA can be described using the following equation:

drug + DNA ↔ drug – DNA

An equation for amperometric titration can be deduced according to [9,10,18,27]

$$\log\left(\frac{1}{[DNA]}\right) = \log(K) + \log\left(\frac{I_{H\text{-}G}}{I_G - I_{H\text{-}G}}\right) \quad (12)$$

Where K is the apparent binding constant, I_G and $I_{H\text{-}G}$ the peak current of the free guest (G) and the complex (H-G), respectively. Under the assumption of diffusion – controlled electron transfer and the complex of drug with DNA (in nucleotide phosphate) is 1:1 association complex, then the plot of log (1 / [DNA]) versus log ($I_{H\text{-}G}$ / (I_G - $I_{H\text{-}G}$)) becomes linear with the intercept of log (K). The binding constant of this complex were evaluated according Eq. (12) and the results are listed in Table I.

Linear range of DNA determination

The decrease in peak current of SIM resulted from the addition of DNA into SIM solution can be employed to determine the concentration of DNA. The peak current of DPV of SIM at -1.388 V was used as the detection signal. Under the optimum experimental condition of Figure 5, the decreases in the DPV peak current were linearly related to DNA concentration in the range of 0 – 10.15 μg mL^{-1} when SIM concentration were fixed at 20.0 ng mL^{-1}, detection limit of DNA was found as 0.0025 μg mL^{-1}. Data of the calibration curves for the proposed method were given in Table II.

Limit of detection (LOD) was estimated by the equation:

LOD=3.3S/m (13)

S: Standart deviation of the intercept, m: Slope of the regression line [28]

The relative standard deviation (RSD) of six experiments performed at DNA concentration level of 1.85 μg mL^{-1} was % 1.4, indicating that the

TABLE I

Binding constant of SIM – DNA complex calculated from the results of voltammetry at pH 7.1

Complex	Cyclic voltammetry, K (M^{-1})	Differential pulse voltammetry, K (M^{-1})
SIM – DNA	1.48 (± 0.15) x 10^4	1.57 (± 0.15) x 10^4

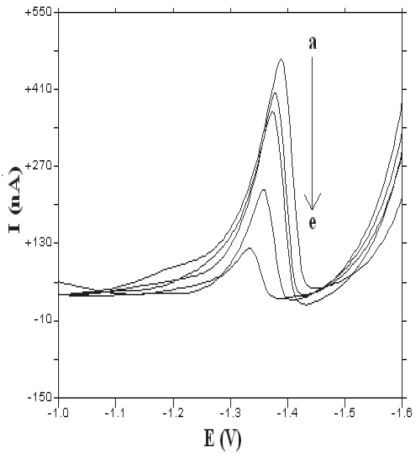


Figure 5

Differential pulse voltammograms of 20.0 ng mL^{-1} SIM in the absence (a) and presence of (b) 1.73 (c) 3.85 (d) 5.15 (e) 7.15 μg mL^{-1} DNA. Step potential (Es) = 0.004 V and pulse amplitude 0.05 V

proposed method can provide a reproducible determination. The results suggested that the proposed method is simple and sensitive hence it can be applied to the determination of many kinds of DNA.

Effect of the ionic strength on the interaction of SIM with DNA

The effect of the ionic strength on the interaction of SIM with DNA using cyclic voltammetry was also investigated to acquire possible binding modes. It has been shown that when a charged ligand is added to a polyelectrolyte solution, its binding constant, K, depends on the total counter ion concentration, $[Na^+]$, as follows[29].

$$d\log K/d\log[Na^+]=\Delta r= -z\Psi \quad (14)$$

Where z is the charge on the ligand molecule, Ψ is the fraction of counterions and Δr is the number of counter ions released upon binding

TABLE II
Data of the calibration curves for the proposed method (n = 7)

Regression equation	y = - 19.189 x + 319.78
Standart error of slope	1.18
Standart error of intercept	1.74
Correlation coefficient (r)	0.9994
Linearity range (DNA concentration)	0 – 10.15 μg mL^{-1}
Number of data points	7
LOD	0.0025 μg mL^{-1}

y = ax + b; y: peak current (nA), x: DNA concentration (μg mL^{-1}), a: Slope, b: Intercept, LOD: Limit of Detection

of the ligand with charge z. The theoretical slope of the log K vs. log[Na^+] relation is -0.88 for a singly charged ligand bound to the B form of DNA in aqueous solutions at 25^{0}C [29]. The plot of log K vs. log[Na^+] was linear with a slope of -0.44. A comparison of the experimentally determined value of the slope with that predicted by theory indicates that electrostatic attractions are less important for the interaction of SIM with DNA.

UV/Vis spectra

Figure 6 showed the UV/Vis absorption spectra of SIM in the absence and presence of different concentrations of DNA. The maximum absorbance of SIM was located around at 230, 238 and 245 nm. It was observed that on the addition of DNA, SIM showed an decrease in molar absorptivity with a red shift of 1 - 6 nm. This hypochromic effect is thought to be due to the interaction between the electronic states of the intercalating chromophore and those of the DNA bases[30]. The SIM solution exhibited hypochromic effect and bathochromic shift in UV/Vis spectra upon binding to DNA, a typical characteristic of an intercalating mode [31].

Based on the variations in the absorbance spectra of SIM upon binding to DNA, the binding constant (K) was calculated according to the equation (15) [32,33]. The absorbance at 238 nm was used to calculate the binding constant (K) in UV/Vis spectra.

$$\frac{A_0}{A - A_0} = \frac{\varepsilon_G}{\varepsilon_{H\text{-}G} - \varepsilon_G} + \frac{\varepsilon_G}{\varepsilon_{H\text{-}G} - \varepsilon_G} \frac{1}{K\,[DNA]} \qquad (15)$$

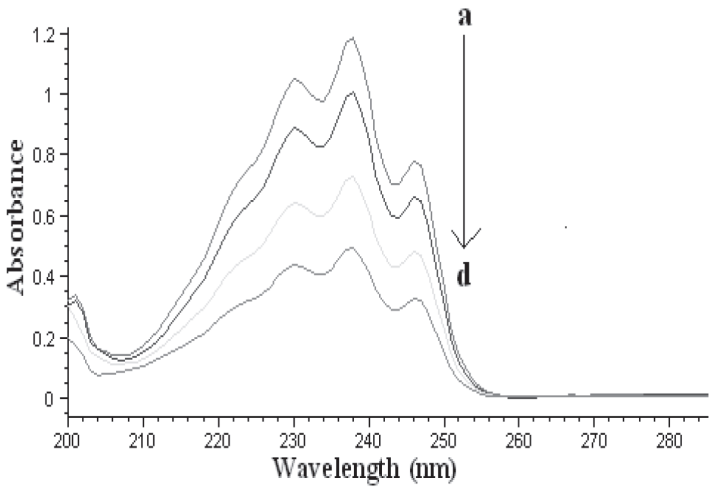


Figure 6
UV-VIS absorption spectra of 1.0 x 10-5 mol L^{-1} SIM in the absence (a) and presence of (b) 1.73 (c) 3.85 (d) 5.15 μg mL^{-1} DNA

where A_0 and A are the absorbances of drug in the absence and presence of DNA, ε_G and $\varepsilon_{H\text{-}G}$ are the absorption coefficients of drug and its complex with DNA, respectively. According to Eq. (15), the plot of A_0 / (A – A_0) versus 1 / [DNA] was constructed using the data from the absorbance titrations and a linear fitting of the data yielded the binding constant (K) 1.41 x 10^4 M^{-1} for SIM - DNA. These results are close to that from voltammetry (Table I).

Conclusions

In this study, the interaction of SIM with DNA was studied by cyclic voltammetry, differential pulse voltammetry and UV/Vis spectroscopy. The binding of SIM to DNA resulted in a series of changes in the electrochemical behavior and spectra characteristics. Upon binding of SIM to DNA, absorption spectra of SIM showed hypochromic effect and bathochromic shift. From these experimental results, it could be affirmed that the interaction of SIM with DNA through intercalative mode. In addition that the effect of the ionic strength on the interaction of SIM with DNA

was investigated and It was found that electrostatic interactions were less important for the interaction of SIM with DNA. The results demonstrate that the electrochemical and spectroscopic methods are available, and offers great promise for study of the mechanism of the interaction between DNA and targeting compounds. The electrochemical method developed here offers important advantages of low detection limit, low cost, sensitivity, rapid and easy applicability.

Summary

The interaction of simvastatin (SIM), a hipolipidemic drug, with human genomic DNA has been investigated by cyclic voltammetry (CV), differential pulse voltammetry (DPV) and UV/Vis spectroscopy. As a result of the interaction of SIM with human genomic DNA, a considerable decrease in the SIM peak currents and a hypochromic and bathochromic shift in the maximum adsorption bands of SIM was observed. The changes in the electrochemical and spectral characteristics of SIM indicated SIM bind to DNA by intercalative mode. Binding constants were determined using voltammetric and spectroscopic data. In addition that the effect of ionic strength on the interaction support the intercalation of SIM into the DNA double helix. These studies are valuable for a better understanding SIM – DNA interaction, which should be important into the determination of therapeutic efficacy of the drug and design of new DNA targeted drug in future.

Key Words: Simvastatin, Human genomic DNA, Electrochemistry, UV/Vis spectroscopy

Özet

İnsan Genomik DNA ile Simvastatinin etkileşmesinin incelenmesi

Bir hipolipidemik ilaç olan simvastatinin insan genomik DNA ile etkileşmesi dönüşümlü voltametri (CV), differansiyel puls voltametrisi (DPV) ve UV/Vis spektroskopisi yöntemleri kullanılarak incelenmiştir.

Simvastatinin insan genomik DNA ile etkileşmesi sonucunda simvastatinin pik akımında önemli bir azalmayla birlikte maksimum absorpsiyon bandlarında hipokromik ve batokromik etki gözlenmiştir. Simvastatinin elektrokimyasal ve spektral özelliklerindeki değişiklikler DNA ile interkalasyon bir şekilde etkileştiğini belirtmektedir. Voltametrik ve spektroskopik veriler kullanılarak bağlanma sabitleri tayin edilmiştir. Ayrıca etkileşme üzerine iyonik şiddetinin etkisi simvastatinin DNA çift sarmalına interkalasyonunu desteklemektedir. Bu çalışmalar ilacın tedavi edici etkisinin belirlenmesi açısından önemli olan simvastatin – DNA etkileşiminin daha iyi anlaşılması ve ilerde yeni DNA hedefli ilaçların tasarlanması açısından değerlidir.

Anahtar Kelimeler: Simvastatin, İnsan Genomik DNA, Elektrokimya, UV/Vis spektroskopisi

REFERENCES

1. Todd, P. A., Goa, K. L.: Simvastatin - a Review of Its Pharmacological Properties and Therapeutic Potential in Hypercholesterolemia, Drugs, 40, 583-607 (1990).
2. Sobal, G., Sinzinger, H.: Effect of simvastatin on the oxidation of native and modified lipoproteins, Biochem Pharmacol, 70, 1185-1191 (2005).
3. Pedersen, T. R., Tobert, J. A.: Benefits and risks of HMG-CoA reductase inhibitors in the prevention of coronary heart disease - A reappraisal, Drug Safety, 14, 11-24 (1996).
4. Gotto, A. M.: Review of primary and secondary prevention trials with lovastatin, pravastatin, and simvastatin, American Journal of Cardiology, 96, 34f-38f (2005).
5. Lambert, B., Lepecq, J. B.: "DNA-Ligand Interactions, From Drugs to Proteins", New York (1986).
6. Porschke, D.: "DNA-Ligand Interactions, Specifity and Dynamics of Protein-Nucleic Acid Interactions", New York (1986).
7. Singh, M. P., Joseph, T., Kumar, S., Bathini, Y., Lown, J. W.: Synthesis and Sequence-Specific DNA-Binding of a Topoisomerase Inhibitory Analog of Hoechst-33258 Designed for Altered Base and Sequence Recognition, Chem Res Toxicol, 5, 597-607 (1992).
8. Pasternack, R. F., Gibbs, E. J., Villafranca, J. J.: Interactions of Porphyrins with Nucleic-Acids, Biochemistry-Us, 22, 2406-2414 (1983).
9. Palecek, E., Kolar, V., Jelen, F., Heinemann, U.: Electrochemical Analysis of the Self-Complementary B-DNA Decamer D(Ccaggcctgg), Bioelectroch Bioener, 23, 285-299 (1990).
10. Chu, X., Shen, G. L., Jiang, J. H., Kang, T. F., Xiong, B., Yu, R. Q.: Voltammetric studies of the interaction of daunomycin anticancer drug with DNA and analytical applications, Anal Chim Acta, 373, 29-38 (1998).
11. Feng, Q., Li, N. Q., Jiang, Y. Y.: Electrochemical studies of porphyrin interacting with DNA and determination of DNA, Anal Chim Acta, 344, 97-104 (1997).
12. Marrazza, G., Chianella, I., Mascini, M.: Disposable DNA electrochemical sensor for hybridization detection, Biosens Bioelectron, 14, 43-51 (1999).

13. Marrazza, G., Chiti, G., Mascini, M., Anichini, M.: Detection of human apolipoprotein E genotypes by DNA electrochemical biosensor coupled with PCR, Clin Chem, 46, 31-37 (2000).
14. Miller, S. A., Dykes, D. D., Polesky, H. F.: A Simple Salting out Procedure for Extracting DNA from Human Nucleated Cells, Nucleic Acıds Res, 16, 1215-1215 (1988).
15. Reichmann, M.E., Rice, S.A., Thomas, C.A., Doty, P.: A Further Examination of the Molecular Weight and Size of Desoxypentose Nucleic Acid, J Am Chem Soc, 76, 3047–3053 (1954).
16. Laviron, E., Roullier, L., Degrand, C.: A Multilayer Model for the Study of Space Distributed Redox Modified Electrodes .2. Theory and Application of Linear Potential Sweep Voltammetry for a Simple Reaction, J Electroanal Chem, 112, 11-23 (1980).
17. Wang, S. F., Peng, T. Z., Yang, C. F.: Electrochemical determination of interaction parameters for DNA and mitoxantrone in an irreversible redox process, Biophys Chem, 104, 239-248 (2003).
18. Carter, M. T., Rodriguez, M., Bard, A. J.: Voltammetric Studies of the Interaction of Metal-Chelates with DNA .2. Tris-Chelated Complexes of Cobalt(Iii) and Iron(Ii) with 1,10-Phenanthroline and 2,2'-Bipyridine, J Am Chem Soc, 111, 8901-8911 (1989).
19. Komorsky-Lovric, S., Nigovic, B.: Electrochemical characterization of simvastatin by abrasive stripping and square-wave voltammetry, J Electroanal Chem, 593, 125–130 (2006).
20. Bard, J., Faulkner, L. R.: "Electrochemical methods Fundamentals and Applications", Wiley: New York (1980).
21. Laviron, E.: Adsorption, Autoinhibition and Autocatalysis in Polarography and in Linear Potential Sweep Voltammetry, J Electroanal Chem, 52, 355-393 (1974).
22. Laviron, E.: General Expression of the Linear Potential Sweep Voltammogram in the Case of Diffusionless Electrochemical Systems, J Electroanal Chem, 101, 19-28 (1979).
23. Anson, F. C.: Evidence for Adsorption of Cobalt(3)-)Ethylenedinitrilo)Tetraacetate at Platinum Electrodes, Anal Chem, 36, 520-& (1964).
24. Wu, L.L., Zhou, J.Z., Luo, J., Lin, Z.H.: Oxidation and adsorption of deoxyribonucleic acid at highly ordered pyrolytic graphite electrode, Electrochim Acta, 45, 2923-2927 (2000).
25. Kalanur, S. S., Katrahalli, U., Seetharamappa, J.: Electrochemical studies and spectroscopic investigations on the interaction of an anticancer drug with DNA and their analytical applications, J Electroanal Chem, 636, 93-100 (2009).
26. Gao, X. X., Yao, X. R.: "Polarographic Catalytic Wave of the Element of Group", Pt. Science Press: Beijing, China (1977).
27. Carter, M. T., Bard, A. J.: Voltammetric Studies of the Interaction of Tris(1,10-Phenanthroline)Cobalt(Iii) with DNA, J Am Chem Soc, 109, 7528-7530 (1987).
28. International Conference on Harmonisation of Technical Requirements for Registration of Pharmacueticals for Human Use, Validation of Analytical Procedures: ICH Harmonised Tripartite Guideline (2005).
29. Record, M. T., Anderson, C. F., Lohman, T. M.: Thermodynamic Analysis of Ion Effects on Binding and Conformational Equilibria of Proteins and Nucleic-Acids - Roles of Ion Association or Release, Screening, and Ion Effects on Water Activity, Q Rev Biophys, 11, 103-178 (1978).
30. Fukuda, R., Takenaka, S., Takagi, M.: Metal-Ion Assisted DNA-Intercalation of Crown Ether-Linked Acridine-Derivatives. Journal of the Chemical Society-Chemical Communications, 1028-1030 (1990).

31. Takenaka, S., Ihara, T., Takagi, M.: Bis-9-Acridinyl Derivative Containing a Viologen Linker Chain - Electrochemically Active Intercalator for Reversible Labeling of DNA, Journal of the Chemical Society-Chemical Communications, 1485-1487 (1990).

32. Dang, X. J., Nie, M. Y., Tong, J., Li, H. L.: Inclusion of 10-methylphenothiazine and its electrochemically generated cation radical by beta-cyclodextrin in water plus methanol solvent mixtures, J Electroanal Chem, 437, 53-59 (1997).

33. Dang, X. J., Nie, M. Y., Tong, J., Li, H. L.: Inclusion of the parent molecules of some drugs with beta-cyclodextrin studied by electrochemical and spectrometric methods, J Electroanal Chem, 448, 61-67 (1998).