

Flexibility of Na,K-ATPase Secondary Structure upon Drug-Protein Interaction

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The enzyme Na⁺, K⁺-ATPase is an integral membrane protein which transports sodium and potassium cations against an electrochemical gradient. The transport of Na⁺ and K⁺ ions is connected to an oscillation of the enzyme between the two conformational states, the E₁ (Na⁺) and the E₂ (K⁺) conformations. The enzymatic activity of ATPase is largely affected by different ligands complexation. This review reports the effects of several drugs such as AZT (anti-AIDS), cis-Pt (antitumor), aspirin (anti-inflammatory) and vitamin C (antioxidant) on the stability and secondary structure of Na,K-ATPase *in vitro*. Drug-enzyme binding is mainly through H-bonding to the polypeptide C=O and C-N groups with two binding constants K_{1(AZT)} = 5.30 × 10⁵ M⁻¹ and K_{2(AZT)} = 9.80 × 10³ M⁻¹ for AZT and one binding constant for K_{cis-Pt} = 1.93 × 10⁴ M⁻¹, K_{aspirin} = 6.45 × 10³ M⁻¹ and K_{ascorbate} = 1.04 × 10⁴ M⁻¹ for cis-Pt, aspirin and ascorbic acid. The enzyme secondary structure was altered from that of α-helix 19.8% (free protein) to almost 22-26% and the β-sheet from 25.6% to 18-22%, upon drug complexation with the order of induced stability AZT > cis-Pt > ascorbate > aspirin.

Keywords: ATPase, Drug-Enzym, Binding mode, Binding constant, Secondary structure, UV-Vis and FTIR

INTRODUCTION

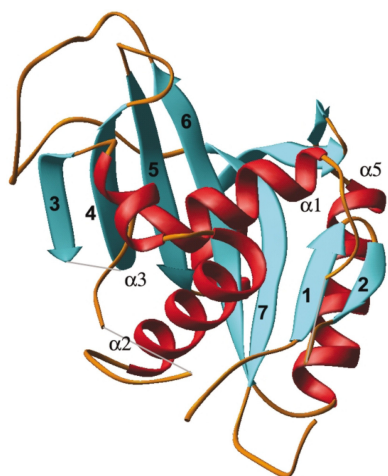
The Na⁺,K⁺-ATPase is an integral membrane protein which transports sodium and potassium cations against an electrochemical gradient. The transport of Na⁺ and K⁺ ions is presumably connected to an oscillation of the enzyme between the two conformational states, the E₁ (Na⁺) and the E₂ (K⁺) conformations [1-5]. The E₁ and E₂ states have different affinities for ligand interaction. It is also reasonable to assume that the translocation process influenced by the enzyme-ligand complexation, which can induce significant changes in the protein configuration possibly near the ligand binding sites. Therefore, in this report we review the effects of AZT, cis-Pt, aspirin and vitamin C complexation on the ATPase secondary structure and protein stability.

3'-Azido-3'-deoxythymidine (AZT), a nucleobase reverse transcriptase inhibitor, was the first drug approved by Food and Drug Administration (FDA) for the treatment of AIDS and is still widely used in combination with other antiviral drugs [6]. The use of highly active antiretroviral drugs against HIV-1 virus dramatically decreased the mortality related to AIDS in developed countries [7]. AZT is considered to be converted into 3'-azido-3'-deoxythymidine-5'-triphosphate (AZTTP) by cellular kinases [8]. The inhibitory mechanism of AZTTP has been extensively studied and it has been concluded that the incorporation of drug into the DNA chain results in inhibition of DNA replication by chain termination [9-14]. Aspirin belongs to the nonsteroidal anti-inflammatory drugs and it is used extensively as a painkiller. It exerts its anti-inflammatory effect through selective acetylation of serin 530 on prostaglandin H₂ synthase [15]. Cis-diamminedichloroplatin(II) (cis-Pt) is an anticancer drug,

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which bind DNA *via* two adjacent guanine bases and causes cell death [16,17]. Vitamin C participates in several oxidation-reduction processes and it is a powerful antioxidant, which prevents DNA damage [18].

In this review the FTIR and UV-Vis spectroscopic data of the Na,K-ATPase interactions with AZT, cis-Pt, aspirin and vitamin C in aqueous solutions at physiological condition are reported. Spectroscopic data regarding the drug binding mode, drug binding constant and the effect of drug complexation on the protein stability and secondary structure are compared here.



Na⁺,K⁺-ATPase

EXPERIMENTAL

Materials

Na,K-ATPase was prepared from guinea pig kidney in 10% (w/v) sucrose containing 25 mM TrisHCl (pH 7.5) and 0.1 mM EDTA [19]. The enzyme specific activity was measured to be 5.6 unit/mg, using standard methods [19-21]. The protein concentration was about 0.53-1.09 mg ml⁻¹ with the purity of more than 90% [19]. D₂O (99.8% atom D) was from Aldrich Chemical Co., and used as supplied. AZT, cis-Pt, aspirin and vitamin C were from Sigma Chemical Co., and used as supplied.

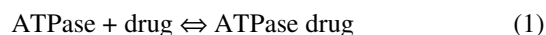
Preparation of Stock Solution

The enzyme was in 10% (w/v) sucrose containing 25 mM Tris-HCl (pH 7.5) and 0.1 mM EDTA [19]. Before spectral

measurement, the Na,K-ATPase was brought into complex with ouabain by incubation for 30 min at 37 °C in a H₂O solution, containing 25 mM Tris-HCl (pH 7.5), 100 mM NaCl, 2 mM MgCl₂, 2 mM ATP and 1 mM ouabain solution. For D₂O solution, the Na,K-ATPase was suspended in heavy water, with 25 mM Tris-DCI (pD 7.5), 100 mM NaCl, 2 mM MgCl₂, 2 mM ATP and 1 mM ouabain at 5 °C for 2 h, 6 h and 24 h (to complete the isotopic D/H substitution). The ouabain binding to Na,K-ATPase and the related inhibition have been recently reported [21,22]. The solutions of AZT, cis-Pt, aspirin and vitamin C of 0.0002 mM to 2 mM were prepared by dissolving drug in double distilled water. In the final step, the solution of drug was added dropwise to protein solution with constant stirring to ensure the formation of homogeneous solution and to attain the desired drug concentrations of 0.0001 to 1 mM. It should be noted that the low drug concentrations of 0.1 μM to 1 μM are in range of the physiological concentrations, while 1 mM exceeds above physiological content. Equal volumes of drug and Na,K-ATPase solutions were incubated for 2 h before spectral measurements. The deuterated samples were prepared in D₂O solution in a similar fashion as for H₂O solutions. The pH or pD of the solution was adjusted to 6.8-7.4.

UV-Vis Absorption Spectra

The absorption spectra were recorded on a Perkin Elmer Lambda 40 spectrophotometer, using various drug concentrations (2 μM to 1 mM) and ATPase concentration of 0.05% (0.5 mg ml⁻¹). To calculate the drug-protein binding constant, the data are treated according to the following equations:



$$K = [\text{ATPase:drug}]/[\text{ATPase}] [\text{drug}] \quad (2)$$

drug = AZT, cis-Pt, aspirin and ascorbate anion

The values of the binding constants K were obtained from the protein absorption at 270 nm according to the method described by published methods [23,24] where the bindings of various ligands to hemoglobin were described. For weak binding affinities the data were treated using linear reciprocal plots based on

$$\frac{1}{A - A_0} = \frac{1}{A_\infty - A_0} + \frac{1}{K(A_\infty - A_0)} \cdot \frac{1}{C_{\text{ligand}}} \quad (3)$$

where, A_0 is the absorption of protein at 270 nm in the absence of ligand, A_∞ is the final absorption of the ligated-protein and A is the recorded absorption at different ligand concentrations. Thus, the double reciprocal plot of $1/(A - A_0)$ vs. $1/C_{\text{ligand}}$ is linear and the binding constant (K) can be estimated from the ratio of the intercept to the slope [23,24].

FTIR Spectroscopic Measurements

Infrared spectra were recorded on a BOMEM DA3-0.02 Fourier transform infrared spectrometer, equipped with a nitrogen cooled HgCdTe detector and a KBr beam splitter. Solution spectra were recorded on hydrated film, using AgBr windows with resolution of 2 cm^{-1} and 100-500 scans. The water subtraction was carried out with 100 mM NaCl solution used as a reference at pH 6.5-7.5. A good subtraction was achieved as shown by a flat baseline around 2200 cm^{-1} where the water combination mode is located. The difference spectra [(enzyme solution + drug solution) - (enzyme solution)] were generated using the lipid and polypeptide antisymmetric and symmetric C-H stretching bands [25-27] located at $2900\text{-}2800 \text{ cm}^{-1}$ as internal standard. The lipid and protein C-H stretching vibrations exhibit no spectral changes upon drug interaction and they are cancelled on spectral subtraction. The accuracy of this subtraction method was tested by using several control samples with the same protein or drug concentration that resulted into a similar flat baseline formation. However, a similar absorption scale was used, for the spectra of the free enzyme and its drug complexes, as well as for the difference spectra produced. This method allowed us to obtain meaningful spectral differences between the free protein and its drug complexes at different drug concentrations. Such spectral differences were used here, in order to characterize the nature of the drug-enzyme interaction.

Determination of Protein Secondary Structure

The determination of the secondary structure of the Na,K-ATPase and its drug complexes is carried out mainly on the basis of the procedure described by Byler and Susi [26]. The protein secondary structure is determined from the shape of the amide I band, located at $1650\text{-}1660 \text{ cm}^{-1}$. Fourier self-

deconvolution and second derivative resolution enhancement are applied, so as to increase the spectral resolution in the region of $1700\text{-}1600 \text{ cm}^{-1}$. The second derivative was produced by using a number of points of convolution such as 11 and 13. The resolution enhancement that results from the self-deconvolution and the second derivative is such that the number and the position of the bands to be fitted are determined. In order to quantify the area of the different components of amide I contour, revealed by self-deconvolution and second derivative, a least-square iterative curve-fitting is used to fit the Gaussian line shapes to the spectra between 1700 and 1600 cm^{-1} . Before curve-fitting was done, a straight baseline passing through the ordinates at 1700 and 1600 cm^{-1} was subtracted. The baseline is then modified again by the least-square curve-fitting, which allowed for a horizontal baseline to be adjusted as an additional parameter in order to obtain the best fit. It is known that no meaningful curve-fitting can be performed by simple examination of the original infrared spectra that is why the self-deconvolution procedure has to be carried out first.

The curve-fitting was done using a number of 50 simulations. The resulting curve fitted is analysed as follows. Each Gaussian band is assigned to a secondary structure according to the frequency of its maximum; α -helix ($1649\text{-}1660 \text{ cm}^{-1}$); β -sheet ($1615\text{-}1637 \text{ cm}^{-1}$); turn ($1660\text{-}1680 \text{ cm}^{-1}$); random coil ($1638\text{-}1648 \text{ cm}^{-1}$) and β -antiparallel ($1680\text{-}1692 \text{ cm}^{-1}$) [26]. The area of all the component bands assigned to a given conformation are then summed and divided by the total area. The number obtained is taken as the proportion of the polypeptide chain in that conformation. These assignments are according to the previous values determined theoretically [25] and experimentally [26]. The accuracy of this method is tested on several proteins of the known conformational contents (through X-ray diffraction analysis), such as cytochrome c (α -helix 49%) and RNase A (α -helix 27%) [28,29], that resulted in 2-3% differences. The data treatments of protein conformational analysis are also reported in our previous publications [30,31].

RESULTS AND DISCUSSION

FTIR Spectra of Na,K-ATPase

The infrared spectrum of the Na,K-ATPase in H_2O

solution exhibits a strong and broad band centered at 3300 cm^{-1} due to the amide A (N-H stretching mode) and three sharp bands located at $2950\text{--}2850\text{ cm}^{-1}$ related to the protein and lipid C-H symmetric and antisymmetric stretching vibrations [27]. The lipid ester carbonyl stretching appears as a band with medium intensity at 1730 cm^{-1} [27]. A strong and broad band at $1651\text{--}1653\text{ cm}^{-1}$ is due to the amide I (mainly polypeptide carbonyl stretching mode), while amide II (C-N stretch and N-H bending modes) appears as a band with medium intensity at 1550 cm^{-1} [25-27] (Fig. 1). A band with medium intensity at 1518 cm^{-1} is attributed to the tyrosine amino acid side-chain vibration [32-35].

In the deuterated samples (D_2O solution), the amide I band at 1651 cm^{-1} , showed minor shift towards lower frequencies at 1648 cm^{-1} , while the amide II band at 1550 cm^{-1} exhibited major shift towards a lower frequency at 1538 cm^{-1} , upon protein deuteration (spectrum not shown). The tyrosine band at 1518 cm^{-1} showed no major shifting, upon enzyme deuteration. From the intensity ratio variations of the amide II band, the protein deuteration process was monitored and there were no major changes between the rate of deuteration after, 2 h, 6 h and 24 h. This indicates that the isotopic substitution (D/H) is completed 2 h after the enzyme has been dissolved with D_2O solution.

From the intensity ratio measurements of the amide II band at 1538 cm^{-1} , it was shown that, the accessible part of the protein for deuteration is less than 50%, indicating that the protein unordered portion is less than 50% of the total conformation. However, to calculate the exact amount of the random coil structure, the spectrum of the Na,K-ATPase in D_2O was subtracted from that of the corresponding H_2O solution, which resulted in a broad positive feature centered at 1647 cm^{-1} in the difference spectrum (spectrum is not shown) [36]. This band is attributed to the protein random coil structure, with its integrated intensity of $38 \pm 2\%$ relative to that of the amide I band at 1651 cm^{-1} . This is in agreement with the amount of random coil structure (37%), previously reported through CD spectroscopic measurement [5]. However, to calculate the regular part of the protein secondary structure, infrared self-deconvolution and second derivative resolution enhancement with curve fitting procedures were performed (see experimental section) and the results are shown in Fig. 2 and Table 1.

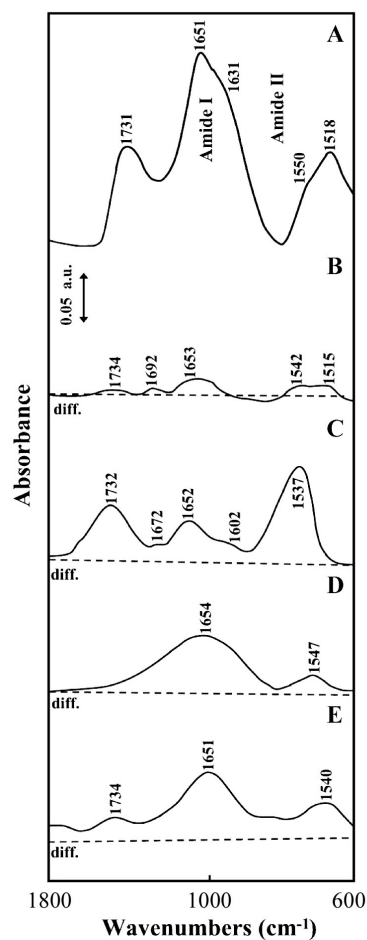


Fig. 1. FTIR spectra (top first curve) and difference spectra [(enzyme solution + drug solution) - (enzyme solution)] (bottom four curves) for the free Na,K-ATPase and its drug complexes in aqueous solution at physiological pH with 1 mM drug concentration in the region of $1800\text{--}1500\text{ cm}^{-1}$: (A) Free Na/K-ATPase (H_2O), (B) AZT-ATPase = 1mM, (C) cis-Pt-ATPase = 1 mM, (D) Aspirin-ATPase = 1 mM, (E) Ascorbate-ATPase = 1 mM.

Drug-ATPase Complexes

The drug interaction leads to major intensity increases for protein amide I band at 1651 and amide II band at 1550 cm^{-1} with positive features at $1650\text{--}1655\text{ cm}^{-1}$ and $1537\text{--}1547\text{ cm}^{-1}$ (Fig. 1, difference spectra for AZT-, cis-Pt-, aspirin- and ascorbate-ATPase complexes). This is due to the drug

Flexibility of Na,K-ATPase Secondary Structure

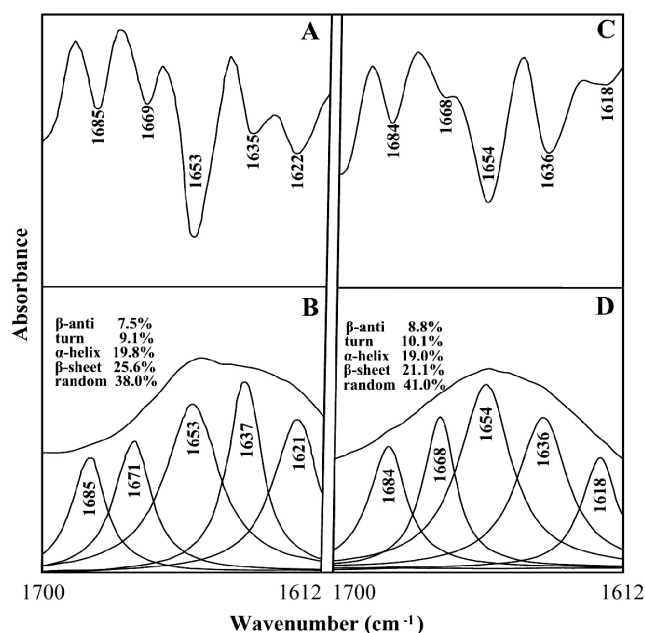


Fig. 2. Second derivative resolution enhancement and curve-fitted amide I region (1700-1612 cm^{-1}) and secondary structure determination of the free Na,K-ATPase (curves A and B) and its drug complexes (curves C and D) in H_2O solution with 1 mM AZT concentration.

interaction (via H-bonding) with protein C=O and C-N groups. Drug interaction with lipid C=O group also was observed for AZT-, cis-Pt- and ascorbate-ATPase complexes, which is evident by a positive feature at 1732-1734 cm^{-1} in the difference spectra (Fig. 1). Similarly, the participation of tyrosine residue in AZT-ATPase complex was observed by the presence of a positive feature at 1515 cm^{-1} (Fig. 1, difference spectra of AZT-ATPase).

A quantitative analysis of the secondary structure of the Na,K-ATPase and its drug complexes in H_2O and D_2O solutions is presented in Fig. 2 and Table 1. The free enzyme in H_2O and D_2O contained α -helix 19.8% (16.8%); β -sheet 25.6% (22.5%); β -antiparallel 7.5% (9.8%); turn 9.1% (10.9%); and random coil 38% (the values in parentheses are for D_2O solution) [36]. Drug interaction results in protein secondary structural changes from that of the α -helix 19.8% (16.8); β -pleated 25.6% (22.5%); turn 9.1% (10.9%); β -antiparallel 7.5% (9.8%) and random 38% in the free Na,K-ATPase to that of the α -helix 19.0-25.9%; β -pleated 17.7-23.2%; turn 8.0-10.1%; β -antiparallel 2.2-8.8% and random 41-45%, in the drug-ATPase complexes in H_2O solution (Fig. 2 and Table 1, Fig. 2 shows curve-fitting for AZT-ATPase adduct).

The furanose ring in the free AZT moiety shows C2'-

Table 1. Secondary Structure Determination for the Free Na/K-ATPase and its Drug Complexes in H_2O at pH 7.2 (the Mean Deviation was $\pm 1.0\%$ to $\pm 2.0\%$ for the Free Enzyme and its Drug Complexes). The Values in Parentheses are from D_2O Solution

Amide I components (cm^{-1})	ATPase free $\text{H}_2\text{O}/(\text{D}_2\text{O})$ (%)	ATPase-AZT 1 mM (%)	ATPase-cis-Pt 1 mM (%)	ATPase-aspirin 1 mM (%)	ATPase-ascorbate 1 mM (%)
1682-1687 β -anti	7.5 \pm 1.0 (9.8 \pm 1.0)	8.8	2.2	4.6	3.3
1668-1670 turn	9.1 \pm 1.0 (10.9 \pm 1.0)	10.1	9.4	8.0	8.1
1653-1655 α -helix	19.8 \pm 1.0 (16.8 \pm 1.0)	19.0	22.2	24.5	25.9
1648 random	(38.0 \pm 2.0)	(41.0)	(43.0)	(45.0)	(45.0)
1614-1637 β -sheet	25.6 \pm 1.0 (22.5 \pm 1.5)	21.1	23.2	17.9	17.7

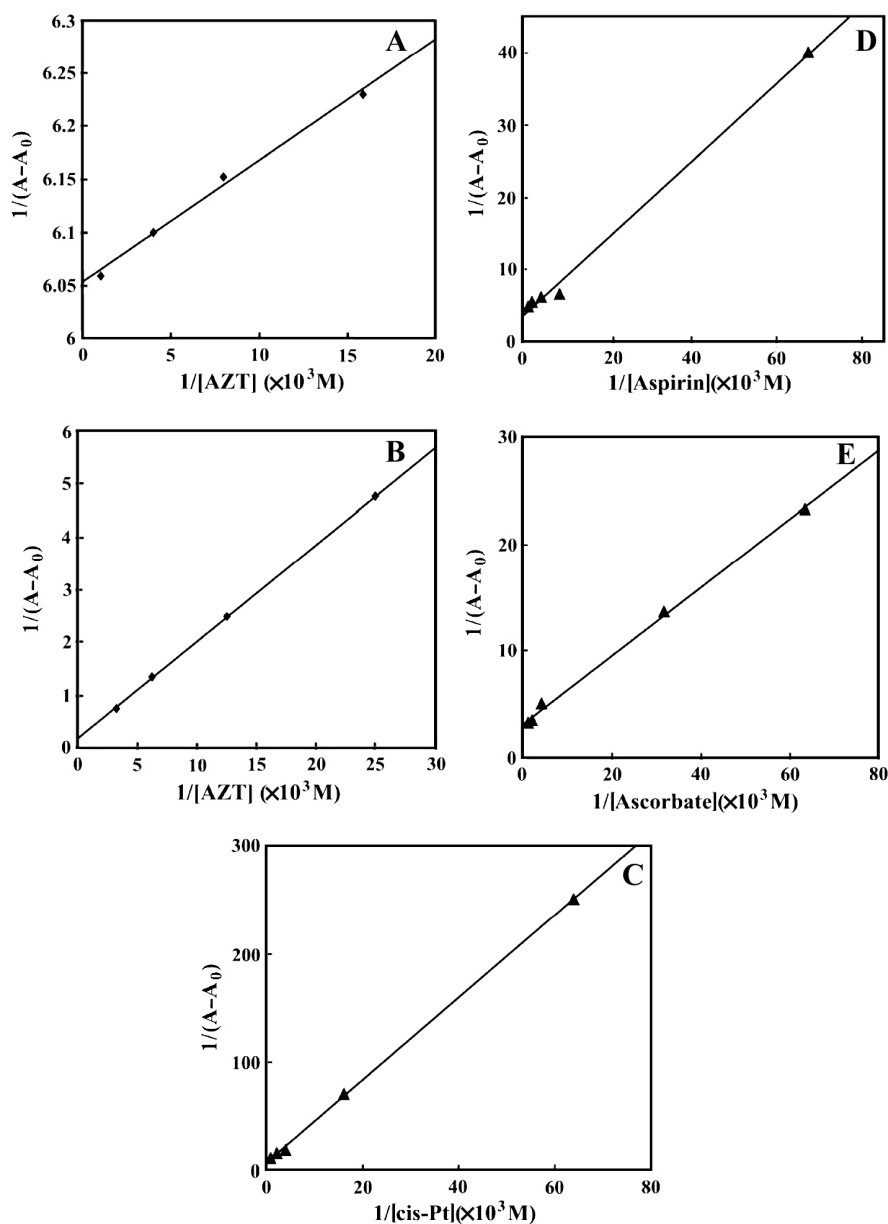


Fig. 3. The double reciprocal plot and the binding constant (K) for drug-ATPase complexes. A_0 is the initial absorption of protein (at 270 nm) and A is the recorded absorption at different drug concentrations: (A) AZT-ATPase ($K_1 = 5.3 \times 10^5 \text{ M}^{-1}$), (B) AZT-ATPase ($K_2 = 9.8 \times 10^3 \text{ M}^{-1}$), (C) cis-Pt-ATPase ($K = 1.93 \times 10^4 \text{ M}^{-1}$), (D) Aapirin-ATPase ($K = 6.45 \times 10^3 \text{ M}^{-1}$), (E) Ascorbate-ATPase ($K = 1.04 \times 10^4 \text{ M}^{-1}$).

endo/anti with marker infrared bands at 892 and 840 cm^{-1} , which is suggested to be a preferred conformation for AZT interaction with HIV-1 [37]. Since no major shifting of these

vibrations occurred and no new bands were observed in the region of 900-800 cm^{-1} , thus the drug sugar moiety remains in the C2'-endo/anti pucker, in these AZT-ATPase complexes

[38]. However, the C=O and C-O stretching vibrations of the aspirin and ascorbate anions showed major shifting upon ATPase interaction, that are indicative of participation of anion C=O and C-O groups in drug-protein complexation [39].

Stability of Drug-ATPase Complexes

The double reciprocal plot of drug concentrations versus the absorption change is shown in Fig. 3. The results show that linearity is noticeable for both low and high values of $1/[\text{drug}]$. Thus, two binding constants are calculated according to equations given in UV-Vis section (Materials and Methods). Strong binding constant of $K_1 = 5.3 \times 10^5 \text{ M}^{-1}$ was obtained from the low values of $1/[\text{drug}]$ (Fig. 3) and a weaker $K_2 = 9.8 \times 10^3 \text{ M}^{-1}$ from the high values of $1/[\text{drug}]$ (Fig. 3). This result reflects the presence of two AZT binding sites on ATPase [38]. In our previous study the interaction between AZT and human serum albumin by affinity capillary electrophoresis showed two bindings with $K_1 = 1.90 \times 10^6 \text{ M}^{-1}$ and $K_2 = 2.1 \times 10^4 \text{ M}^{-1}$ [40]. More recently, one binding with $K = 5.29 \times 10^5 \text{ M}^{-1}$ was observed for AZT with RNase [41]. However, weaker interactions were observed for aspirin, ascorbate and cis-platin drugs adducts with Na,K-ATPase with $K_{\text{aspirin}} = 6.45 \times 10^3 \text{ M}^{-1}$, $K_{\text{ascorbate}} = 1.04 \times 10^4 \text{ M}^{-1}$ and $K_{\text{cis-Pt}} = 1.93 \times 10^4 \text{ M}^{-1}$ [39,42] (Fig. 3). Similarly, one binding constant was observed for aspirin-HSA, cis-Pt-HSA and aspirin-RNase complexes [43-45]. These binding constants are similar to those of the other ligand-protein interactions [46].

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REFERENCES

- [1] K.O. Hakansson, *J. Mol. Biol.* 332 (2003) 1175.
- [2] C. Tanford, *Proc. Natl. Acad. Sci. USA* 79 (1982) 2882 and references cited therein.
- [3] S.M. Gloor, *FEBS Lett.* 412 (1997) 1.
- [4] I.M. Glynn, S.J.D. Karkish, *Annu. Rev. Physiol.* 37 (1984) 13.
- [5] T.I. Gresalfi, B.A. Wallace, *J. Biol. Chem.* 259 (1984) 2622.
- [6] C.C.J. Carpenter, D.A. Cooper, M.A. Fischl, J.M. Gatell, B.G. Gazzard, S.M. Hammer, M.S. Hirsch, D.M. Jacobsen, D.A. Katzenstein, J.S.G. Montaner, D.D. Richman, M.S. Saag, M. Schechtler, R.T. Schooley, M.A. Thompson, S. Vella, P.G. Yeni, P.A. Volberding, *J. Am. Med. Assoc.* 283 (2000) 381.
- [7] M. Rigourad, C. Ehresmann, M.A. Parniak, B. Ehresmann, R. Marquet, *J. Biol. Chem.* 277 (2002) 18611.
- [8] H. Mitsuya, K.J. Weinhold, P.A. Furman, M.H. St. Clair, S.N. Lehrman, R.C. Gallo, D. Bolognesi, D.W. Barry, S. Border, *Proc. Natl. Acad. Sci. USA* 82 (1985) 7096.
- [9] P.S. Kedar, J. Abbotts, T. Kovacs, K. Lesiak, P. Torrence, S.H. Wilson, *Biochemistry* 29 (1990) 3609.
- [10] J.E. Reardon, W.H. Miller, *J. Biol. Chem.* 265 (1990) 20302.
- [11] M. Qui-Fen, I.C. Bathurst, P.J. Barr, G.L. Kenyon, *Biochemistry* 31 (1992) 1375.
- [12] M.S. Chen, R.T. Suttman, E. Papp, P.D. Cannon, M.J. McRobert, C. Bach, W.C. Copeland, T.S.-F. Wang, *Biochemistry* 32 (1993) 6002.
- [13] O. Turriziani, G. Antonelli, F. Focher, F. Bambacioni, F. Dianzani, *Biochem. Biophys. Res. Commun.* 228 (1996) 297.
- [14] H.A. Tajmir-Riahi, *J. Iran. Chem. Soc.* 2 (2005) 78.
- [15] P.J. Loll, D. Picot, M. Garavito, *Nature (London)*, 2 (1995) 637.
- [16] P.M. Takahara, A.M. Rosenzweig, C.A. Frederick, S.J. Lippard, *Nature* 377 (1995) 649.
- [17] A.M.J. Fichtinger-Schepman, J.L. van der Verr, J.H.J. den Hartog, P.H.M. Lohman, J. Reedijk, *Biochemistry* 24 (1985) 707.
- [18] J.M. May, *FASEB J.* 13 (1999) 995.
- [19] L.R. Stepp, M.A. Novakoski, *Arch. Biochem. Biophys.* 337 (1997) 43.
- [20] C. Hegyvary, R.L. Post, *J. Biol. Chem.* 246 (1971) 5234.
- [21] R.L. Post, A.K. Sen, *Methods Enzymol.* 10 (1967) 762.

- [22] C.F.L. Fontes, F.E.V. Lopes, H.M. Scofano, H. Barrabin, J.G. Norby, *Arch. Biochem. Biophys.* 366 (1999) 215 and references therein.
- [23] J.J. Stephanos, *J. Inorg. Biochem.* 62 (1996) 155.
- [24] J.J. Stephanos, S.A. Farina, A.W. Addison, *Biochim. Biophys. Acta* 1295 (1996) 209.
- [25] S. Krimm, J. Bandekar, *Adv. Protein Chem.* 38 (1986) 181.
- [26] D.M. Byler, H. Susi, *Biopolymers* 25 (1986) 469.
- [27] E.V. Brazhnikov, A.B. Chetverin, Y.N. Chirgadze, *FEBS Lett.* 93 (1978) 125.
- [28] E. Goormaghtigh, V. Cabiaux, J.M. Ruyschaert, *Eur. J. Biochem.* 193 (1990) 409.
- [29] G. Vandenbussche, A. Clercx, T. Curstedt, J. Johansson, H. Jornvall, J.M. Ruyschaert, *Eur. J. Biochem.* 203 (1992) 201.
- [30] A. Ahmed, H.A. Tajmir-Riahi, R. Carpentier, *FEBS Lett.* 363 (1995) 65.
- [31] M. Purcell, J.F. Neault, H.A. Tajmir-Riahi, *Biochim. Biophys. Acta* 1478 (2000) 61.
- [32] H. Matsuura, K. Hasegawa, T. Miyazawa, *Spectrochim. Acta* 42A (1986) 1181.
- [33] J.M. Olinger, D.M. Hill, R.J. Jakobsen, R.S. Brody, *Biochim. Biophys. Acta* 869 (1986) 89.
- [34] H. Fabian, C. Schultz, J. Backmann, U. Hahn, W. Saenger, H.H. Mantsch, D. Naumann, *Biochemistry* 33 (1994) 10725.
- [35] T. Yamamoto, M. Tasumi, *J. Mol. Struct.* 242 (1991) 235.
- [36] J.F. Neault, H. Malonga, S. Diamantoglou, R.L. Tepp, R. Carpentier, H.A. Tajmir-Riahi, *J. Biomol. Struct. Dyn.* 20 (2002) 173.
- [37] S. Dijkstra, J.M. Benevides, G.J. Thomas JR, *J. Mol. Struct.* 242 (1991) 283.
- [38] A. Ahmed Ouameur, J.F. Neault, S. Claveau, H.A. Tajmir-Riahi, *Cell Biochem. Biophys.* 42 (2005) 87.
- [39] J.F. Neault, A. Benkiran, H. Malonga, H.A. Tajmir-Riahi, *J. Biomol. Struct. Dyn.* 19 (2001) 95.
- [40] S. Gaudreau, J.F. Neault, H.A. Tajmir-Riahi, *J. Biomol. Struct. Dyn.* 19 (2002) 1007.
- [41] S. Gaudreau, A. Novetta-Dellen, J.F. Neault, S. Diamantoglou, H.A. Tajmir-Riahi, *Biopolymers (Biospectroscopy)* 72 (2003) 435.
- [42] J.F. Neault, A. Benkiran, H. Malonga, H.A. Tajmir-Riahi, *J. Inorg. Biochem.* 86 (2001) 603.
- [43] J.F. Neault, A. Novetta-Delen, H. Arakawa, H. Malonga, H.A. Tajmir-Riahi, *Can. J. Chem.* 78 (2000) 291.
- [44] J.F. Neault, H.A. Tajmir-Riahi, *Biochim. Biophys. Acta* 1384 (1998) 153.
- [45] J.F. Neault, C. Ragi, A. Novetta-Delen, H.A. Tajmir-Riahi, *Cell Biochem. Biophys.* 46 (2006) 27.
- [46] H.A. Tajmir-Riahi, *J. Iran. Chem. Soc.* 3 (2006) 297.

Abbreviations

Na,K-ATPase = Na⁺, K⁺-dependent adenosine triphosphatase; AZT = 3'-azido-3'-deoxythymidine; HIV = human immunodeficiency virus; asp = aspirin; asc = ascorbate; cis-Pt = cis-Pt(NH₃)₂Cl₂; HSA = human serum albumin; FTIR = Fourier transform infrared.