Computational Study of the Structure, the Flexibility, and the Electronic Circular Dichroism of Staurosporine – a Powerful Protein Kinase Inhibitor

Tatyana G. Karabencheva-Christova, Warispreet Singh, and Christo Z. Christov

Department of Applied Sciences, Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, NE1 8ST, United Kingdom

Reprint requests to C. Z. C.; E-mail: christo.christov@northumbria.ac.uk

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This paper is dedicated to the 75th birthday of Prof. Jörg Fleischhauer.

Staurosporine (STU) is a microbial alkaloid which is an universal kinase inhibitor. In order to understand its mechanism of action it is important to explore its structure-properties relationships. In this paper we provide the results of a computational study of the structure, the chiroptical properties, the conformational flexibility of STU as well as the correlation between the electronic circular dichroism (ECD) spectra and the structure of its complex with anaplastic lymphoma kinase.

Key words: Staurosporine; Computational Chemistry; Electronic Circular; Dichroism; Molecular Dynamics; Anaplastic Lymphoma Kinase.

1. Introduction

Staurosporine (STU) is a microbial alkaloid which is a universal kinase inhibitor that has a strong cytotoxic effect on cancer cells [1] and also exhibits antimicrobial, hypotensive activity against yeast and fungi. Moreover, it causes platelet aggregation, inhibition of smooth muscle contraction, and activation of macrophages [2, 3]. STU and its analogues are widespread in nature. The title compound was first isolated from actinomycete *Streptomyces staurosporeus* in 1977 [4] but it also occurs in cyanobacteria and marine invertebrates, including sponges, tunicates, and mollusks [3, 5]. STU belongs to the indolocarbazole family of compounds which is presented in the living organisms only in form of its indolo(2,3-a)carbazoles [6] isomers. STU is a derivative of indolo(2,3-a)pyrrole(3,4-c)carbazole [2, 7]. The biosynthesis of the indolocarbazole ring in STU involves two tryptophan molecules, the sugar moiety from glucose, and methionine [8]. The products of several genes participate in the biosynthetic process [3]. The chemical structure of STU (Fig. 1) can be divided into two units:

i) an aminodesoxy sugar which adopts the boat conformation in the solid state, and is linked to the aglycone through a pair of C–N bonds and

ii) a planar indolocarbazole heterocycle [9].

STU was also chemically synthesized [8] and the structure of STU and its absolute configuration (2′S, 3′R 4′R, 6′R) were determined using X-ray crystallography [10]. Anti-tumour properties of STU have extensively been reported in scientific literature over the past few decades [7, 11, 12]. STU is a highly potent and non-specific inhibitor of various forms of kinases [13], and to date about 337 three dimensional X-ray crystal structures of STU and its analogues exist in the RCSB protein data bank (PDB) (www.rcsb.org) [14]. The crystal structures reveal that STU binds with high affinity to adenosine triphosphate (ATP) binding sites which are usually located between nitrogen and carbon terminal domains of kinases. The indolo(2,3-a)carbazole moiety tends to occupy the hydrophobic adenine-binding pocket while the lactamate group might form hydrogen bonds to the backbone of the hinge segment [3]. The sugar moiety tends to adopt a boat-shaped conformation in the solid state and might participate in hydrophobic interactions as well as in hydrogen bonds within the ribose binding site (Fig. 2) [15]. The high number of hydrogen bonds between the methylamino nitrogen of the glycosidic ring with surrounding residues might also contribute to the binding affinity [3]. Thus STU exhibits binding affinity towards a broad spectrum of

various kinases, and therefore the title compound itself would not be successful as an anti-cancer drug itself because it would also interfere with other non-pathological cellular processes [16]. In order to solve the issue of broad specificity of STU, various forms of STU analogues have been designed (e. g. UNC-01, UNC-02, K25a, and rebeccamycine) which exhibit high level of selectivity against various kinases [9]. Interactions between the STU and the kinases induce structural changes which can be explored by a variety of experimental and computational methods. Electronic circular dichroism (ECD) is a fast and sensitive method for exploring changes in the secondary structure (far UV) and in the tertiary structure (near UV) of proteins and protein-ligand complexes [17, 18]. Experimental ECD curves can be compared to calculated ECD spectra and important insight at the atomic level can be gained [19, 20].

In this paper we present the results of a computational study of the structure, the flexibility, and the electronic circular dichroism of STU followed by a model study of the effect of STU binding [21] on the ECD spectra of an important STU target, anaplastic lymphoma kinase (ALK) [22].

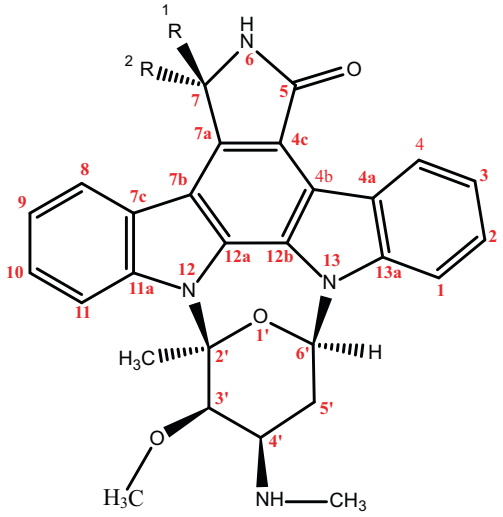

Fig. 1 (colour online). Chemical structure of Staurosporine [10]. STU (R1 = R2 = H) 1 UCN-01 (R1 = OH, R2 = H) 5 UCN-02 (R1 = H, R2 = OHO6).

2. Methods

The structure of STU was taken from the ALK-STU complex available from the protein data bank (PDB) (PDB ID 3LCS.PDB) [15]. The hydrogen atoms were added using GaussView5.0 [23] and the molecule's geometry was optimized at the density functional theory (DFT) level [24, 25] with the B3LYP functional and 6-31+G(d) [26 – 29] basis set. Calculations were performed both in the gas phase and with the polarisable continuum model presenting a hydrophobic environment (dielectric constant 4.0). Subsequently time-dependent density functional (TD-DFT) calculations were carried out for the first 20 singlet states using the same functional and the same basis set. All electronic structure calculations were done using the Gaussian09 code [30]. The ECD spectra were visualized using GaussView5.0. Molecular dynamics simulations were carried out using Gromacs 4.5.3 code [31]. The force field parameters

Table 1. Geometric parameters of STU in the solid state, the optimized values obtained at the B3LYP/6-31+G(d) level in gas phase, the B3LYP/6-31+G(d)-optimized structural parameters in presence of solvent using PCM model, and the averaged one from 100 ns MD simulation.

STU chemical structure	PDB structure numbering	STU PDB structure	STU in gas	STU in solvent	MD averaged structure
Dihedral angles					
4b, 3′, 2′, 4′N4	O6, C22, C21, N4	90	−30.36	−30.23	121
4′N4, 4′, 5′, 13	N4, C23, C24, N3	−160.28	−136.8	−136.31	−141.63
4′, 5′, 6′, 1′	C23, C24, C25, O4	5.35	−8.74	−8.69	9.14
(4′ NH C28), (4′ N4), 6′, 13	C28, N4, C25, N3	−138.22	126.28	127.45	−132.67
(3′ O6 C27), (3′ O6), 12, 11a	C27, O6, N2, C17	−13.03	−11.52	−10.24	8.89
Angles					
5′, 4′, 3′	C24, C23, C22	106.1	108.23	108.31	106.45
2′, 1′, 6′	C21, O4, C25	118.25	121.13	121.07	120.42
H-Bond					
(3′ O6), (4′ N4)	O6, N4	3.09	2.94	2.94	2.79
(4′N4), (4′ N4,H43), (3′ O6)	N4, H43, O6	89	99.5	100.8	74.61

Table 2. Conversion between atomic names numbers of STU according the chemical nomenclature and protein data bank (PDB).

Atom type	Chemical structure	PDB structure
O5	5, O5	1
C8	5	2
N1	6	3
H12	6, H12	4
C9	7	5
H9	7, R1, R2	6
C10	7a	7
C7	4c	8
C6	4b	9
C5	4a	10
C4	4	11
H41	4, H41	12
C3	3	13
H3	3, H3	14
C2	2	15
H2	2, H2	16
C1	1	17
H11	1, H11	18
C20	13a	19
N3	13	20
C19	12b	21
C18	12a	22
C11	7b	23
C12	7c	25
C13	8	25
H13	8, H13	26
C14	9	27
H14	9, H14	28
C15	10	29
H15	10, H15	30
C16	11	31
H16	11, H16	32
C17	11a	33
N2	12	34
C21	2′	35
C26	2′ C26	36
O4	1′	37
C25	6′	38
C24	5′	39
C23	4′	40
N4	4′ N4	41
H42	4′ N4, H42	42
H43	4′ N4, H43	43
C28	4′ NH, C28	44
C22	3′	45
O6	3′ O6	46
C27	3′ O6, C27	47

were calculated using the PRODRG online server (http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg)[32]. The structure was optimized in the gas phase, then solvated using a box with single point charge (SPC) water molecules [33], and finally minimized again. Subsequently a productive molecular dynamics (MD) simulation was run for 10 ns in isothermal-isobaric (NPT) ensemble at 310 K applying the Berendsen thermostat. Protein circular dichroism calculations were carried out using the semiempirical matrix method [34] as implemented on the DICHROCALC server [35] (http://comp.chem.nottingham.ac.uk/dichrocalc/) using the ab initio derived monopoles [36, 37]. The calculations were done as follows:

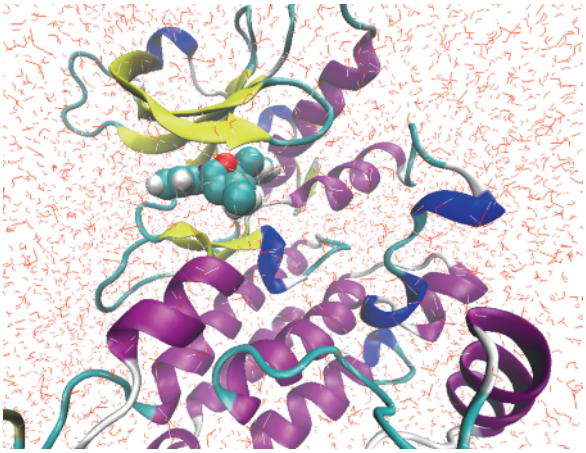

Fig. 2 (colour online). Structure of the complex of anaplastic lymphoma kinase complex with STU (prepared from solvated structure 3LCS.pdb and visualized using VMD [22]).

i) using the crystal structures of the free anaplastic lymphoma kinase (3L9P.PDB), the protein parts from the ALK-STU complex (3LCS.PDB), and ALK-adenosine diphosphate (ALK-ADP) complex (3LCT.PDB) and

ii) using the averaged structures of the protein parts from the 100 ns MD simulations of the free enzyme and the ALK-STU and ALK-ADP complexes [38].

The visualization of the ALK-STU complex was done using the visual molecular dynamics (VMD) program [22].

3. Results and Discussion

Some geometric parameters of potentially more flexible parts of the STU molecule, derived from the quantum-chemically optimized coordinates and crystal structures of STU are presented in Table 1. The structures optimized in the gas phase and those obtained with the solvent model look very similar. More significant differences were found between the minimized structures and the structure in the solid state used as starting points of our geometry optimizations.

Table 3. Distances and dihedral angles between the tryptophan chromophores in the solid state and the free ALK and ALK-Staurosporine complex. The distances are measured between the CD2 atoms from two residues and the dihedral angles between the CD1 and CD3 atoms from the first residue and the CD3 and CD1 atoms from the second residue. The atom names are according PDB nomenclature.

TRP Residues	ALK Distance (Crystal Structure)	ALK Dihedral angle (Crystal Structure)	ALK-STU Distance (Crystal Structure)	ALK-STU Dihedral angle (Crystal Structure)
1295-1320	9.89	37.17	9.85	36.52
1295-1366	11.08	46.67	11.17	46.15
1295-1313	10	26.77	10.06	28.49
1320-1366	8.24	135.64	8.32	136.05
1320-1313	12.37	128.3	12.42	128.66
1366-1313	5.77	34.38	5.82	35.2

Table 4. Distances and dihedral angles between the tryptophan chromophores in the MD-averaged structures of the free ALK and ALK-Staurosporine complex. The distances are measured between the CD2 atoms from two residues and the dihedral angles between the CD1 and CD3 atoms from the first residue and the CD3 and CD1 atoms from the second residue. The atom names are according PDB nomenclature.

TRP Residues	ALK Distance (MD-averaged)	ALK Dihedral angle (MD-averaged)	ALK-STU Distance (MD-averaged)	ALK-STU Dihedral angle (MD-averaged)
1295-1320	9.75	47.25	10.52	−115.01
1295-1366	11.44	59.44	11.26	−136.61
1295-1313	11.63	5.28	8.86	100.16
1320-1366	7.78	−31.41	7.75	62.06
1320-1313	12.46	118.05	11.87	110.7
1366-1313	5.72	18.48	5.85	15.66

This might be due to the fact that the crystal structures sometimes do not correspond to local minima of the free molecule and, therefore, contain some restraints due to the crystal packing [39]. In the quantum-chemically optimized structures an intramolecular hydrogen bond between the amino hydrogen and the methoxy oxygen atoms of the sugar is formed as can be seen by the values for the corresponding distances and angles in Table 1. Such hydrogen bonding interactions might contribute to the binding of STU to kinase molecules.

The correlation between the chemical nomenclature and atom names according PDB is presented in Table 2.

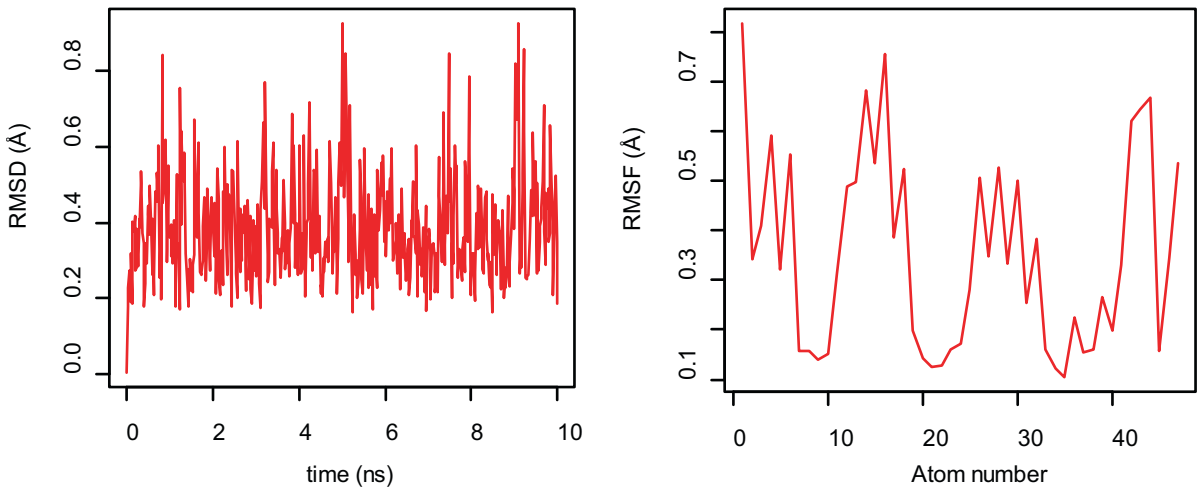

Fig. 3 (colour online). The RMSD and RMSF values of STU.

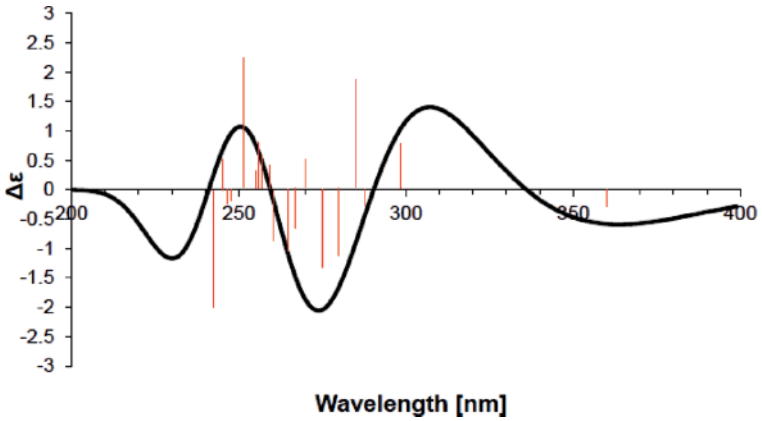

Fig. 4 (colour online). Calculated ECD spectrum of STU at the B3LYP/6-31+G(d) level. The calculated rotational strengths are presented as vertical bars.

Valuable insight into the interactions between STU and kinases can be received by computational modelling and in particular by using classical molecular dynamics simulations which utilize sets of parameters (force fields) for the bonding and the non-bonding interaction and charges for the atoms in a molecule. In order to explore the importance of the conformational flexibility of STU we performed 10 ns MD simulations in a solvent box of SPC water molecules. The geometric parameters of the averaged structure from the MD simulations are also given in Table 1 and the root mean square deviation (RMSD) and the root mean square fluctuation (RMSF) profiles are shown in Figure 3. These values suggest that the different parts of the molecule exhibit different flexibilities and this would have an impact on how STU interacts, accommodates, and adapts within the kinases binding sites.

ECD is routinely applied for determining the absolute configurations of small chiral compounds and often it is the only method of choice (when crystallography and nuclear magnetic resonance are not applicable) [40]. In protein science ECD is used to detect changes in the secondary structure (far UV) and in the tertiary structure (near UV) [14, 41, 42]. The near-UV ECD can indicate delicate structural changes induced by ligand binding, effects of mutation and catalysis. The binding of STU to proteins can be investigated by experimental ECD in combination with calculations. If the ECD calculations based on modelled structures are in good agreement with the experimental spectra they can be used for evaluation of the structures of the kinase-STU complexes as it has been demonstrated for other proteins [19]. An important step for this process is the calculation of the ECD spectrum of the free STU using a relatively accurate method e. g. TD-DFT. The predicted spectrum at B3LYP/6-31+G(d) level is presented in Figure 4. The spectrum is characterized by two minima around 230 nm and 275 nm and two maxima at 250 nm and 310 nm. To the best of our knowledge there is only one available experimental ECD spectrum of STU in the literature [43] but it does not contain the complete set of data, allowing quantitative comparison, therefore we will make only a qualitative comparison. The experimental spectrum is characterized by maxima at around 205 nm, 230 nm, and 300 nm and minima at around 210 nm and smaller one around 265 nm [43]. Besides a red shift the calculated ECD spectrum is in qualitative agreement with its experimental counterpart but further improvement can possibly be achieved using an improved computational model and trying alternative initial structures e. g. optimized at higher quantenmechanical level and/or taken from MD trajectory.

The binding of STU to kinases would affect their ECD spectra in comparison with the free apoenzyme's spectra. This effect can be a result from two contributions:

i) the steric influence due to the changes in protein conformation and in the orientation between protein chromophores upon STU binding and

ii) the effect of the internal ECD of STU and its electrostatic interactions with the protein chromophores (aromatic and peptide ones).

Here we provide qualitative estimation of the first effect using as a model for the complex between STU and ALK, an enzyme of great biomedical and pharma-

ceutical interest [21]. The second one requires more extensive development and non-trivial validations of models and calculations and is an object of an ongoing project. The ECD spectra of the free enzyme and the protein part of the ALK-STU complex were calculated using

i) the respective crystal structures of the free enzyme, and protein parts of ALK-ADP and ALK-STU complexes and

ii) the averaged structures from MD simulations of the free enzyme and the both enzyme-ligand complexes.

The averaged MD structures would allow for some evaluation of the effects of the conformational relaxation and flexibility. In both cases the binding of STU and ADP (as structural effect defined above) influences the ECD spectra (Fig. 5). There are, however, important differences. When the crystal structures are used, the calculated ECD spectrum of the ALK-STU complex is much closer to the ECD spectrum of the free enzyme structure (Fig. 5a). When the conformational flexibility is evaluated in MD simulations, the predicted CD spectrum of the ALK-STU is much more different from the one of the free enzyme and more similar to the one of ALK-ADP (Fig. 5b). The differential spectra of the ALK-STU complex show a much stronger impact of STU binding when the conformational flexibility and relaxation is accounted for (Fig. 5c). A qualitative explanation of this difference could be provided by looking into the mutual orientation between the chromophores in the crystal structures (Table 3) and MD-averaged structures (Table 4) of ALK and ALK-STU complexes. Certainly, this is only a qualitative result, because the experimental ECD spectra of the free ALK and ALK-STU complexes are not yet available, but it is an important step towards a more comprehensive study involving better validation of structural models, monopoles, and experimental ECD measurements.

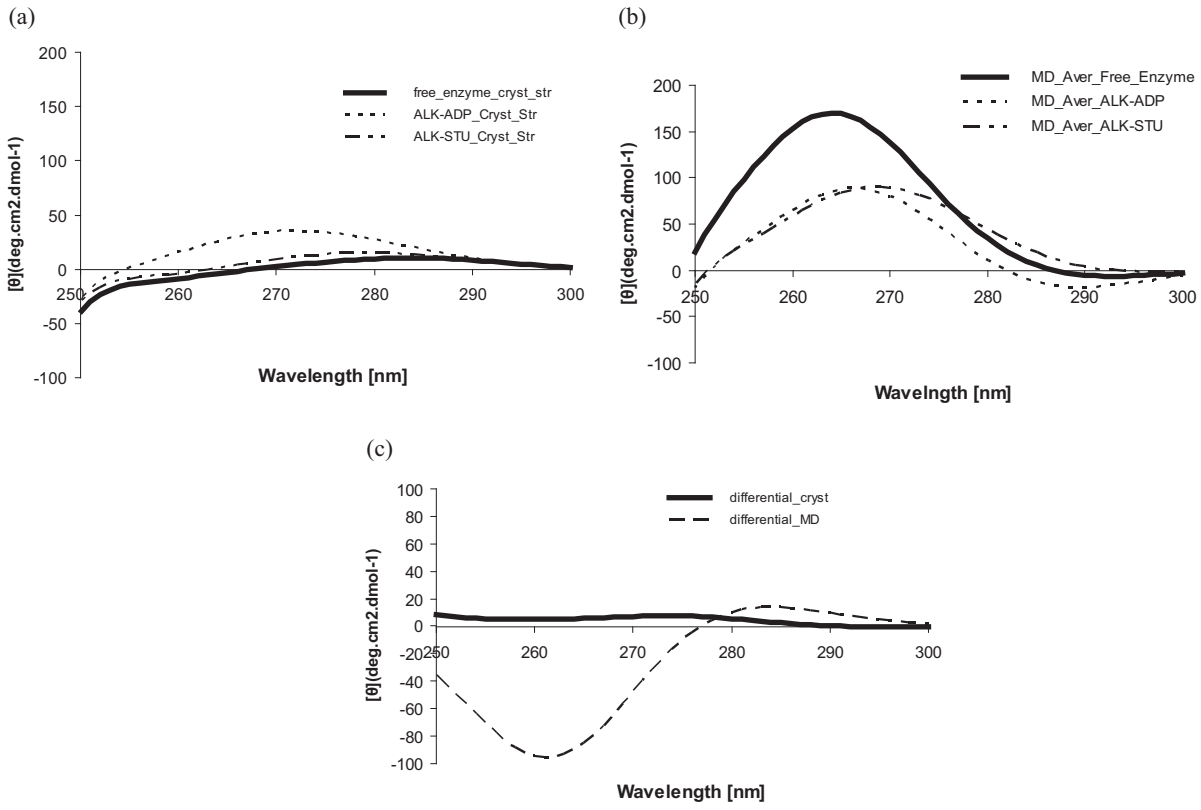

Fig. 5. Calculated near-UV ECD spectra of the free ALK and upon binding of STU and ADP: (a) calculated using the crystal structures; (b) using the averaged structures from MD simulations; (c) the differential spectra for STU calculated from the crystal structures and the MD-averaged structures.

The results presented here suggest that it would be important to investigate more systematically the flexibility of the free STU in solution and in enzyme-ligand complexes. The ECD spectrum of the free STU currently calculated semi-quantitatively could be improved exploring more choices for the initial structure, improving the computational model, and correlating to a more extensive set of experimental data. A combination of experimental ECD spectroscopy and computational modelling would provide sensitive insight into the mechanism of binding of STU to ALK and thus would be useful for drug design.

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