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Interaction Energies for the Purine Inhibitor Roscovitine with Cyclin-Dependent Kinase 2: Correlated Ab Initio Quantum-Chemical, DFT and Empirical Calculations

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Abstract: The interaction between roscovitine and cyclin-dependent kinase 2 (cdk2) was investigated by performing correlated ab initio quantum-chemical calculations. The whole protein was fragmented into smaller systems consisting of one or a few amino acids, and the interaction energies of these fragments with roscovitine were determined by using the MP2 method with the extended aug-cc-pVDZ basis set. For selected complexes, the complete basis set limit MP2 interaction energies, as well as the coupled-cluster corrections with inclusion of single, double and noninteractive triples contributions [CCSD(T)], were also evaluated. The energies of interaction between roscovitine and small fragments and between roscovitine and substantial sections of protein (722 atoms) were also computed by using density-functional tight-binding methods covering dispersion energy (DFTB-D) and the Cornell empirical potential. Total stabilisation energy originates predominantly from dispersion energy and methods that do not account for the dispersion energy cannot, therefore, be recommended for the study of protein–inhibitor interactions. The Cornell empirical potential describes reasonably well the interaction between roscovitine and protein; therefore, this method can be applied in future thermodynamic calculations. A limited number of amino acid residues contribute significantly to the binding of roscovitine and cdk2, whereas a rather large number of amino acids make a negligible contribution.

Keywords: ab initio calculations · cyclin-dependent kinase · density functional calculations · molecular mechanics · roscovitine

Introduction

The transfer of information in a cell is mediated through various linked signalling pathways, leading finally to the control of diverse metabolic processes. Protein kinases play a crucial role in many of these pathways, for example, regulation of the cell cycle, differentiation, membrane transport and secretion of cellular proteins, such as growth hormones.[1,2] A deregulation of cyclin-dependent kinases (cdk) was demonstrated in human primary tumors and human tumor lines.[3] This discovery stimulated interest in cdk and their inhibitors because of their potential applications as anticancer drugs.[4] These cdk enzymes catalyse the transfer of the ATP phosphate to serine and threonine residues located on a protein substrate and, thus, activate other proteins. The activity of cdk2 is regulated by association with regulatory subunits (cyclins) and through phosphorylation by other kinases, which cause conformational changes in the protein so that the correct positioning of a substrate and catalytic residues is achieved.[5,2] The cdk2 associated with cyclin E can promote progress through the G1 phase and, consequently, it forms a complex with cyclin A, which is a necessary condition for entry of a cell into the S phase.

Cdk2 is comprised of 298 amino acid residues and possesses a typical kinase fold containing two lobes: a small N-terminal and a large C-terminal domain. The lobes are connected through a single polypeptide strand. The active site

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that binds ATP or purine-like inhibitors is situated between the lobes in a deep cleft, and serves as a primary target for drug design. It consists of three sections named according to the ATP moieties that it binds: the purine binding site, the phosphate-group pocket and the sugar pocket. The X-ray structures of cdk2 and its complexes with different inhibitors have been published[6–10] and the number of these continues to rise.

The inhibition of cdk2 plays a key role in drug development programmes addressing such different pathological conditions as inflammation, autoimmunity, cancer, and cardiovascular and neurodegenerative diseases.[4,11] Consequently, many pharmaceutical companies are interested in drugs that regulate the activity of specific eukaryotic protein kinases. Both experimental and theoretical approaches are used for this purpose. Various computational studies describing the thermodynamic properties of binding protein with inhibitor by using molecular mechanics-generalised Born solvation area (MM-GBSA) methodology,[12,13] the molecular simulation of this process[14] and the docking process exist.[15] All these studies were based on empirical potentials, which is understandable considering the size of the system under investigation. The applicability of empirical potential should, however, be carefully tested; our recent studies have shown its limitations in the context of interaction with DNA bases,[16] the hydrophobic core of proteins[17] and various types of interaction in proteins.[18]

The aim of the present study is to investigate the interaction of cdk2 with the inhibitor roscovitine. Our interest in roscovitine is due to its potential as a promising drug candidate in cancer therapy through blocking cell cycle transitions in cancerous cells by cdk1/2 inhibition. Interaction of cdk2 with roscovitine is very complex and is controlled by not only hydrogen bonding, but also by other energetic contributions, such as dispersion, electrostatic and charge-transfer. An accurate description of all these energetic contributions is difficult and requires high-level ab initio quantum-chemical correlated calculations. These calculations should be also applied in the design of a selective inhibitor with higher activity. An essential condition for such designs is a full understanding of the nature of the stabilisation of the ligand in the active site. Investigation will be focused on not only the physical nature of stabilisation (i.e., the role of various energy contributions, such as hydrogen bonding, stacking, etc.), but also on the steric nature (i.e., the role of various parts of the protein cavity in stabilising inhibitor). Thus, the main difference between previous studies and this present one concerns the non-empirical ab initio quantum-chemical approach, which properly describes the nature of the interaction between roscovitine and protein. We stress again that previous results were based on empirical potential and, therefore, might not be sufficiently accurate.

Strategy of calculations: Cdk2 interacts with roscovitine non-covalently. Of the non-covalent interactions, the most important role is played by hydrogen-bonding, electrostatic, charge-transfer and dispersion interactions. The role of hydrogen bonds is well recognised and their theoretical description is quite straightforward. In fact, almost any empirical, semiempirical and nonempirical methods can describe hydrogen bonding. Much less is known about the role of dispersion energy and originally it was thought to be of low importance. Only recently it was shown that the dispersion energy plays an important role in not only stabilising structures of DNA and proteins, but also in, for example, stabilising an intercalator in DNA. As well as dispersion interaction, charge-transfer also plays an important role in the interaction of protein and inhibitor. A theoretical description of this energy term is also difficult and empirical potential does not incorporate this term at all. Thus, it is necessary to evaluate the interaction of cdk2 with roscovitine at a high theoretical level by embracing all energy terms. Such calculations are impractical for large proteins consisting of several thousands of atoms. For this reason, the whole protein was fragmented into smaller systems consisting of one or a few amino acids. The high-level ab initio correlated calculations performed for these smaller complexes are used as benchmark data for testing the performance of the empirical potential. It is evident that to perform the molecular dynamics simulations of the present system (necessary for the description of dynamic properties) the empirical potential must be used.[19] Besides this, the calculated stabilisation energies allow us to estimate the importance of various sections of the protein cavity in the stabilisation of inhibitor. Here, the accuracy of the calculations is crucial, as stabilisation can originate from hydrogen bonding as well as from stacking, and the balanced description of various energy terms is extremely difficult.

Calculations of cdk2 with roscovitine were performed as follows. Firstly, empirical potentials that are used standardly for protein simulation were applied. This methodology is successful, but is associated with several problems. The potential is pairwise additive and the non-additivity plays a role if systems are polar or charged. Further, interaction energy is comprised of a sum of electrostatic, dispersion and repulsion contributions, which means that the charge-transfer term is completely missing. Thus, secondly, the fast approximative ab initio method that is free of the problems mentioned above is applied. In our laboratory we use the self-consistent charge density-functional tight-binding method augmented empirically for London dispersion energy (SCC-DFTB-D).[20] This method yields excellent results for DNA base pairs and DNA fragments, complexes of intercalator with DNA, as well as for amino acid pairs and protein fragments. The method is very fast and enables single-point calculations to be performed for systems containing several thousands of atoms, and for smaller systems, molecular dynamics simulations can be performed. Finally, the benchmark data for smaller model systems generated by accurate ab initio correlated calculations were used for testing empirical potential and SCC-DFT-D results.

Here, we focus on the interaction energy of the protein–ligand complex with the aim of understanding the nature of its stabilisation and quantification of the role of dispersion

contribution. Interaction energy is determined as a sum of interaction energies of representative fragments containing amino acid(s) and ligand, as well as directly for the whole protein–ligand complex.

Geometries, fragmentation: The crystal structure of protein cyclin-dependent kinase 2 with roscovitine (Figure 1) with 2.4 Å resolution was used.[9] Figure 2 shows a schematic

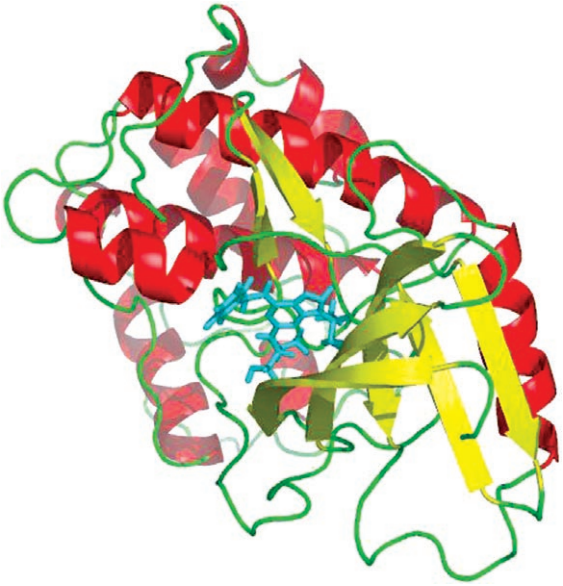

Figure 1. Secondary structure of cdk2 with roscovitine.

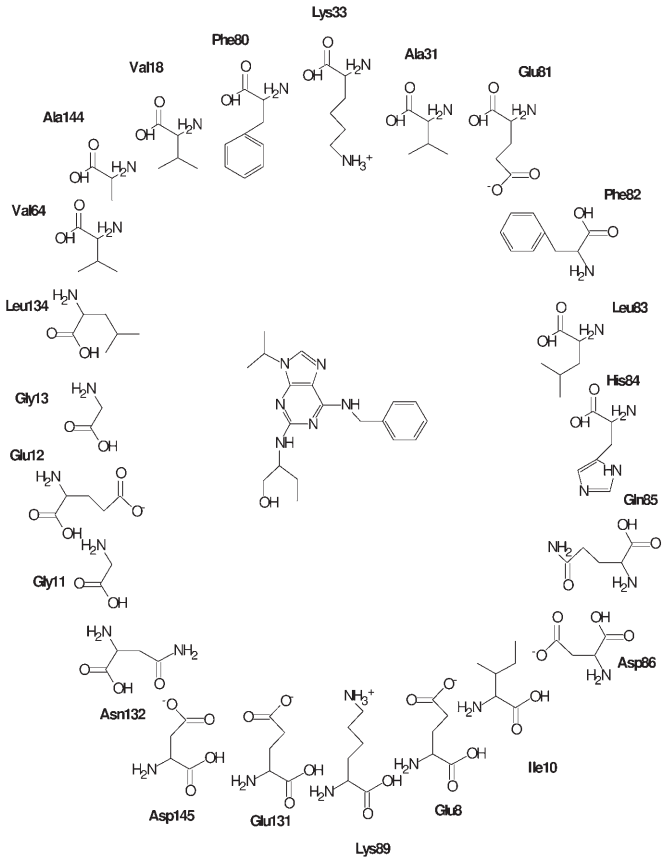

Figure 2. Schematic view of roscovitine (centre) and the key amino acids.

view of roscovitine and some of the key amino acids under consideration. The resolution of the crystal structure of roscovitine with cdk2 is rather high, but it is sufficient for the present computations. The system studied contains protein and ligand and is very complex. To understand the nature of stabilisation, high-level correlated ab initio calculations should be performed. These calculations are, however, impractical for the system considered, whose size should be reduced by, for example, fragmenting the protein into constituent amino acids. The method of fragmentation is not standard and several problems regarding biochemical relevancy should be overcome. In constructing representative fragments, we considered only complexes of specific amino acids directed towards roscovitine, as it is known that only the nearest groups have significant influence on the interaction energy. The amino acid charges were adjusted to pH 7, as the crystal structure considered was analysed under these conditions. A problem occurred for the interactions of roscovitine and the protein backbone, due to strong π–π interaction between peptide bonds and the purine aromatic rings of roscovitine. The protein backbone was, therefore, cut at the C^{α}–N bond and the peptide bond was maintained. During partitioning we considered only amino acids and crystal water molecules located within 5 Å from roscovitine. We created 14 fragmented complexes containing one or more amino acids and roscovitine: glutamic acid 8 (E8); isoleucine 10 (I10); valine 18 (V18); alanine 31 (A31); lysine 33 and aspartic acid 145 (K33D145); valine 64 (V64); phenylalanine 80 (F80); glutamic acid 81, phenylalanine 82 and leucine 83 (E81F82L83); leucine 83, histidine 84 and glutamine 85 (L83H84Q85); glutamine 85, aspartic acid 86 and lysine 86 (Q85D86K89); glutamine 131 and asparagine 132 (Q131N132); leucine 134 (L134); alanine 144 (A144); glycine 11, glutamic acid 12 and glycine 13 (G11E12G13). All complexes are shown in Figure 3 and are labelled according to the amino acid(s) included. Missing hydrogen atoms in these fragmented complexes were added by using InsightII and optimised by the gradient quantum-chemical optimisation at the B3LYP/6–31G** level; positions of heavy atoms were fixed. The molecules of water presented in the crystal structure were also included to improve the description of interaction between roscovitine and a protein fragment. Although all amino acid fragments are neutral, E8 bears a negative (−1) charge, and the substituents of the K33D145 fragment have +1 and −1 charges, respectively. The same is true for Q85D86K89, in which the D86 and K89 substituents have a −1 and +1 charge, respectively.

Because the partitioning of protein is associated with several uncertainties, it is recommended to calculate the interaction energy for as large a portion of the protein as possible. The model system consisting of 722 atoms was obtained

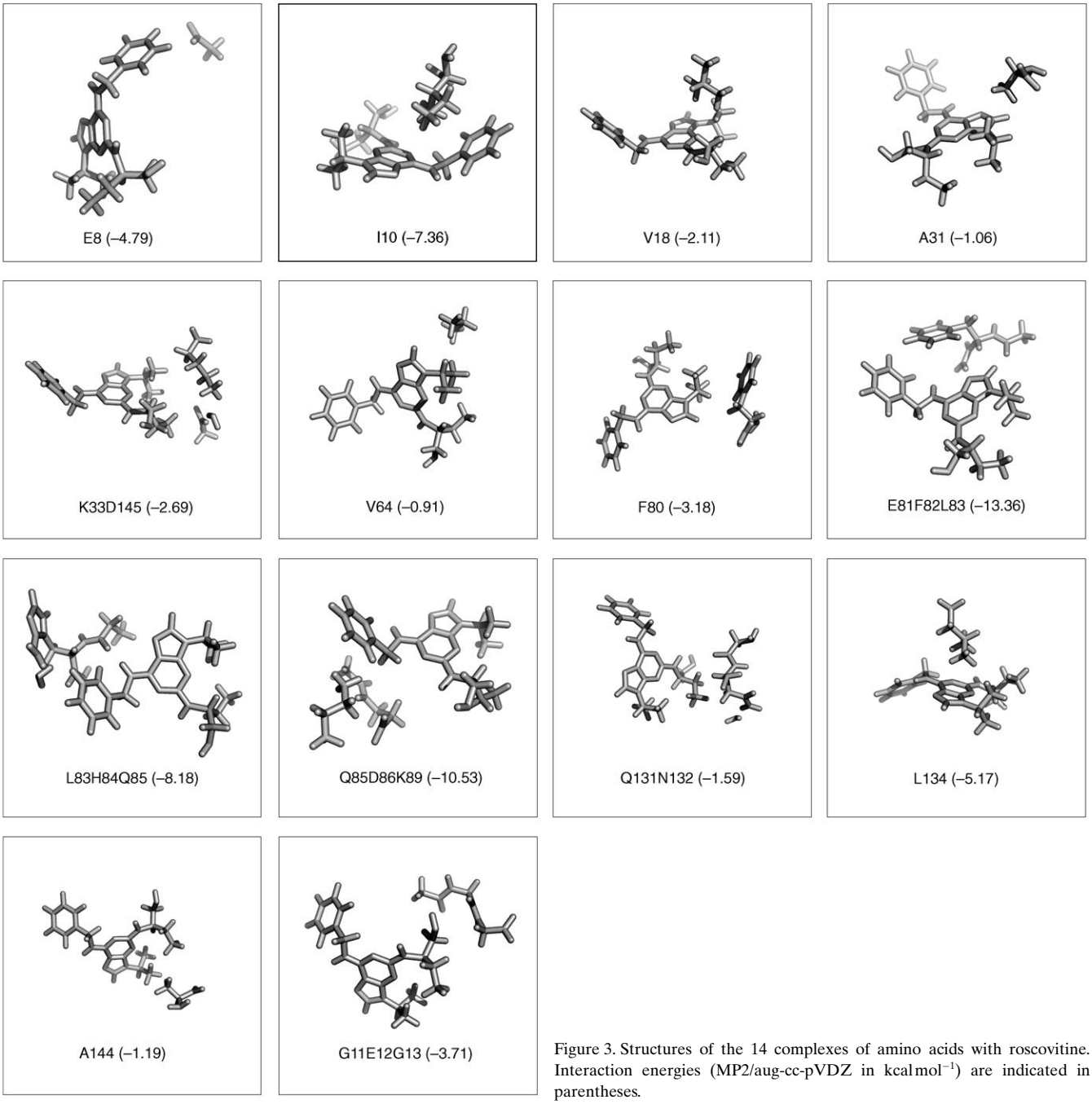

Figure 3. Structures of the 14 complexes of amino acids with roscovitine. Interaction energies (MP2/aug-cc-pVDZ in kcal mol^{-1}) are indicated in parentheses.

by considering all amino acids within a distance of 8 Å from roscovitine. The complex with roscovitine is shown in Figure 4. The positions of missing hydrogen atoms were optimised by using the semiempirical quantum-chemical PM3 method.

Computational Methods

The interaction energies between ligand and fragments of protein (or whole protein) include an important contribution from London dispersion energy. It is, therefore, appropriate to consider only those methods that cover the dispersion energy. We have shown recently[21] that accurate interaction energies of various types of intermolecular complexes (hydrogen-bonded, stacked, T-shaped) are obtained at the coupled-cluster singles and doubles theory with perturbational triples corrections [CCSD(T)] level by using the complete basis set (CBS) limit.

CBS interaction energies are obtained by extrapolating the total energies of supersystem and subsystems.[22] The first rational basis set is the aug-cc-pVDZ one and extrapolations are, thus, performed from the aug-cc-pVDZ and aug-cc-pVTZ energies. The coupled-cluster calculations [CCSD(T)] at the CBS limit (ΔE[CCSD(T)/CBS] for the present complexes is clearly impractical, therefore, we approximated their values by using Equation (1)

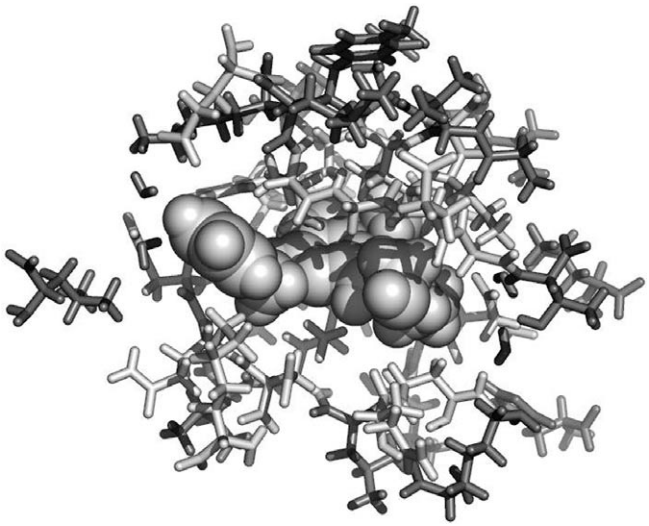

Figure 4. Structure of the protein cavity of cdk2 containing roscovitine. The roscovitine is represented in a ball-and-stick conformation.

$$\Delta E[\mathrm{CCSD(T)/CBS}] = \Delta E(\mathrm{MP2})/\mathrm{CBS} + \Delta E[\mathrm{CCSD(T)-MP2}]/(\mathrm{DZ+P}) \quad (1)$$

in which ΔE(MP2)/CBS stands for the MP2 CBS stabilisation energy and the latter term gives the difference between the CCSD(T) and MP2 stabilisation energies determined by using a medium basis set of the DZ+P quality. The evaluation of the ΔE-[CCSD(T)/CBS] limit by using this equation is based on the assumption that the difference between the CCSD(T) and MP2 interaction energies is less dependent on the quality of the basis set than the CCSD(T) and MP2 energies.[22]

The CCSD(T) and MP2 CBS calculations described are extremely time consuming (e.g., computations of fragment I10 with roscovitine at the MP2 and CCSD(T) levels of theory take about two weeks of CPU time by using a Pentium 4 3 GHz) and were, therefore, performed for only selected complexes. In all remaining cases, the interaction energies were determined at the MP2/aug-cc-pVDZ level. Interaction energies were systematically corrected for the basis set superposition error by using the function counterpoise method of Boys and Bernardi.[23] MP2 calculations with the present basis set would be very time consuming, so we replaced them by resolution of identity (RI)-MP2 calculations. It was shown recently[24] that absolute and relative RI-MP2 and MP2 energies differ marginally, although the former method is by one order of magnitude faster.

Density functional theory (DFT) methods are very popular as they are efficient and enable the study of extended complexes. However, the DFT methods fail to describe the dispersion energy and, thus, their use in biomolecular studies is limited. Here, we demonstrate this by performing DFT/B3LYP/6–31G** calculations. Furthermore, the approximative SCC-DFTB-D method with empirical dispersion term was used.

We used the Cornell empirical potential[19] by using the parm99 parameter set. For the 14 fragmented complexes the RESP HF/6–31G* charges were adopted, whereas for the large model cluster (consisting of 722 atoms) the standard atomic charges from the Cornell library were used.

Results and Discussion

Table 1 shows the interaction energies of the 14 complexes determined by using various theoretical procedures. The ab initio nonempirical results will be discussed first. Extrapolation to the MP2 CBS limit was performed for complexes I10, F80 and L134 and passing from aug-cc-pVDZ to aug-cc-pVTZ yields a non-negligible enlargement of stabilisation energy. The CBS limit stabilisation energies are 10, 9 and 9% larger than the respective aug-cc-pVDZ values. The CCSD(T) correction term was determined for slightly reduced complexes (F80 and L134), giving 0.41 and 0.70 kcal mol^{-1}, respectively, that is, it was slightly repulsive. From the values mentioned above it is clear that we can confidently use the RI-MP2/aug-cc-pVDZ stabilisation energies. Therefore, the remaining discussion will be based solely on these.

It is evident from the data in the first column of the Table 1 that by far the largest stabilisation energy was found for the E81F82L83 cluster. By investigating the structure of the complex (Figure 3) we found two short hydrogen-bonded contacts (2.35 Å): between the amino-group hydrogen of L83 and the N7 nitrogen of the purine ring of roscovitine, and between the carbonyl-group oxygen of the E81 peptide bond and the C8–H hydrogen of the purine ring of roscovitine (2.14 Å). Both contacts are undoubtedly connected by strong hydrogen bonds. Important stabilisation also comes from π–π interactions between the phenyl ring of roscovitine and the phenyl ring of F82. The stabilisation energy of the Q85D86K89 cluster is also large (>10 kcal mol^{-1}) and arises from the interaction between charged subunits D86 ($q=-1$) and K89 ($q=+1$) and neutral roscovitine. Figure 3 shows the existence of the close contact between the NH_3^+ group of K89 and the phenyl-ring group of roscovitine. The cluster L83H84Q85 is characterised by a moderately strong stabilisation energy (8.18 kcal mol^{-1}) that stems from a very short hydrogen bond (1.83 Å)

Table 1. Interaction energies [kcal mol^{-1}] for the 14 fragmented complexes (see Figure 3), determined at the MP2, B3LYP, DFTB, DFTB-D and Amber levels.

	Interaction energy				
Complex	MP2/aug-cc-pVDZ	B3LYP/6–31G**	DFTB	DFTB-D	Amber
E8	−4.79	−3.86	−2.03	−2.47	−1.88
I10	−7.36 (-7.76, −8.08)[a]	1.44	−1.09	−7.57	−7.14
V18	−2.11	2.1	0.18	−2.91	−2.8
A31	−1.06	2.9	0.37	−1.84	−1.6
K33D145	−2.69	1.94	0.14	−3.18	−1.82
V64	−0.91	0.41	0.03	−1.09	−1.02
F80	−3.18 (−3.35, −3.47, 0.41)[b]	0.9	0.07	−3.14	−3.19
E81F82L83	−13.36	−6.12	−4.39	−9.78	−9.04
L83H84Q85	−8.18	0.98	−2.53	−8.57	−8.54
Q85D86K89	−10.53	−5.02	−2.56	−12.02	−7.98
Q131N132	−1.59	1.69	0.67	−2.13	−0.98
L134	−5.17 (−5.42, −5.64, 0.7)[b]	2.68	−0.19	−5.28	−5.52
A144	−1.19	0.96	−0.05	−2.06	−1.6
G11E12G13	−3.71	−1.53	−1.25	−3.35	−4.26
sum	−65.83	−0.53	−12.63	−65.39	−57.36

[a] Numbers in parentheses correspond to MP2/aug-cc-pVTZ and MP2 CBS interaction energies, respectively. [b] Numbers in parentheses correspond to MP2/aug-cc-pVTZ and MP2 CBS interaction energies, and to the CCSD(T) correction term, respectively.

between the oxygen of the carboxyl group of L83 and the hydrogen of the N^6-amino group of roscovitine. Further stabilisation also originates in the π–π interactions between two peptide bonds of L83, H84 and Q85 and the phenyl ring of roscovitine. Stabilisation energies of the three complexes I10, L134 and F80 are 7.36, 5.17 and 3.18 kcal mol^{-1}, respectively, and are due partially to strong C–H–π interactions. In the case of I10, dispersion interaction between the side chain of I10 and the phenyl and purine aromatic rings of roscovitine also contribute to the stability of the complex. The E8 and G11E12G13 complexes have moderately high stabilisation energies (4.79 and 3.71 kcal mol^{-1}, respectively). Stabilisation of the anionic complex E8 is due to interaction between the aromatic phenyl ring of roscovitine and the negatively charged carboxylic group of glutamic acid 8. K33D145, V18, Q131N132 and A31 complexes possesses only weak stabilisation energies (2.69, 2.11, 1.59 and 1.06 kcal mol^{-1}, respectively). The remaining complex (V64) exhibits only negligible stabilisation, less than 1 kcal mol^{-1}.

The total stabilisation energy of roscovitine and the 14 neighbouring fragments is rather larger (~66 kcal mol^{-1}), however, the contributions of single amino acid–roscovitine interactions differ considerably. Five complexes (E81F82L83, Q85D86K89, L83H84Q84, I10 and L134) contribute greatly (about 68%) to total stabilisation. On the other hand, six fragments (V18, A31, K33D145, V64, Q131N132 and A144) contribute less than 14% to total stabilisation (the remaining contribution to stabilisation is from complexes E8, F80 and G11E12G13). This observation is slightly surprising and clearly indicates that selected sections of the protein cavity are much more significant for inhibition than others. Similarly, some regions of the cavity have almost no effect on inhibitor stabilisation. We can speculate about the importance of these findings in the light of the preparation of new (more active) inhibitors. Probably the most efficient way to increase stabilisation would be to increase the binding activity of those amino acid residues that contribute negligibly to the overall stability. Here, we investigated the interaction of cdk2 with inhibitor. In the future, we will study the interaction with modified roscovitine inhibitors. The mutations should be reflected in not only different interaction energies, but also in altered binding affinities modelled by the change in free energy of complexation. The resulting theoretical values could be then compared with existing experimental data, such as SAR values.

The segmentation of protein into amino acid fragments is justified by evaluating the interaction energy for the larger cluster. Figure 5 shows three partial clusters E81F82L83, L83H84Q85 and Q85D86 as well as the composed system E81F82L83H84Q85D86. To make the MP2/aug-cc-pVDZ calculation feasible we removed the side chains from all amino acids, as well as from the N^6 and N^2 roscovitine substituents. From Table 2 we can see that the sum of the interaction energies of the three partial clusters amounts to −20.51 kcal mol^{-1}, whereas the interaction energy of the whole system is −19.28 kcal mol^{-1}. The small difference between these values fully justifies the fragmentation of the protein.

Table 2. Interaction energies [kcal mol^{-1}] for three separate fragmented clusters and the composed system (see Figure 5), determined at the MP2 level.

Complex	MP2/aug-cc-pVDZ
E81F82L83	−11.01
L83H84Q85	−7.25
Q85D86	−2.26
sum of the three fragments	−20.51
E81F82L83H84Q85D86	−19.28

The MP2/aug-cc-pVDZ calculations for the present clusters are demanding and raise the question of whether a simpler quantum-chemical procedure can be applied. This concerns firstly the DFT methods, which are popular within the biochemical community. From the data shown in Table 1, however, we see that the B3LYP/6–31G** values are dramatically different from the correlated MP2/aug-cc-PVDZ data: the sum of all 14 MP2 interaction energies is −65.8 kcal mol^{-1}, whereas the sum of the B3LYP interaction energies is −0.53 kcal mol^{-1}. The huge difference of 65 kcal mol^{-1} is due to the lack of dispersion energy in the B3LYP treatment. Evidently, the B3LYP procedure fails completely and cannot be used for the study of predominantly noncovalent protein–inhibitor interactions. DFT/B3LYP calculations were performed with the 6–31G** basis

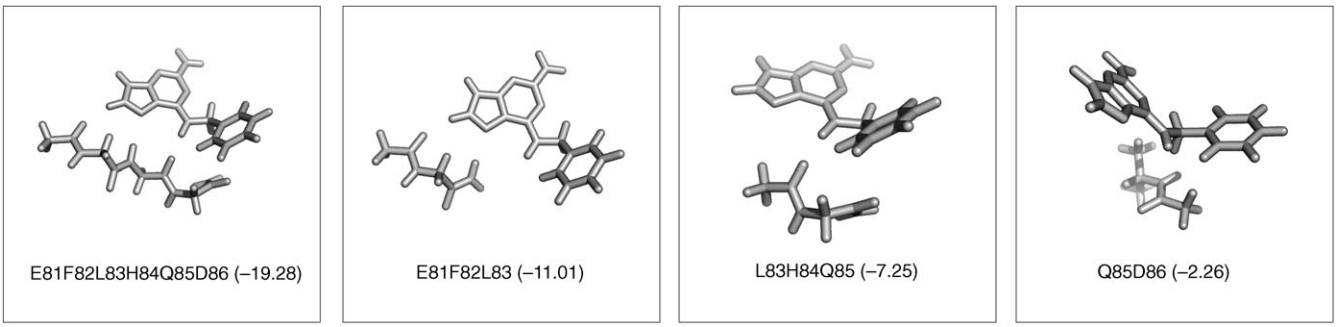

Figure 5. Structures of fragments of the protein backbone. Interaction energies (MP2/aug-cc-pVDZ in kcal mol^{-1}) are indicated in parentheses.

set. The DFT calculations are known to be less sensitive than the MP2 method to the quality of the basis set. We have shown recently[25] that the B3LYP procedure yields very similar stabilisation energies for amino acid pairs, for which the 6–31G** and cc-pVTZ basis set were applied.

Comparison of B3LYP and MP2 results can indicate the role of π–π interactions, which are governed by London dispersion energy. From the data in Table 1 it is evident that practically all of the complexes investigated are stabilised mainly by dispersion energy. The anionic complex E8 represents other example. As expected, the electrostatic contribution (which is properly described in the B3LYP procedure) is dominant and, consequently, the B3LYP and MP2 values differ by less than 1 kcal mol^{-1}. A similar situation occurs in the G11E12G13 cluster, for which the B3LYP and MP2 values basically agree. In all other cases, the differences between these energies were large. The largest difference was found for the L83H84Q84, L134, I10 and E81F82L83 clusters. In each of these clusters we can find an important π–π stacking motif between either a peptide bond and an aromatic ring, an aromatic ring and an aromatic ring or even an aliphatic chain and an aromatic ring.

The surprising role of dispersion energy, demonstrated in the previous paragraph by comparison of MP2 and B3LYP stabilisation energies, can be confirmed by investigating the stabilisation energies originating from the DFTB and DFTB-D procedures. These values differ by the empirical London dispersion energy alone: the sum of all 14 stabilisation energies is 12.6 and 65.4 kcal mol^{-1}, respectively. The latter value is practically identical with the MP2 value, whereas the former value is strongly underestimated and is close to the B3LYP value. The same is true for the comparison of stabilisation energies for single complexes. As for DNA base pairs, DNA base pair–intercalator, and amino acid pairs, in the present case the DFTB-D procedure mimics surprisingly well MP2 interaction energies, whereas B3LYP (and all other standardly used functionals) fails.[25]

After joining all 14 fragments into one "shell", the DFTB and DFTB-D stabilisation energies of the "shell" with the roscovitine amount to 8.8 and 56 kcal mol^{-1}, respectively. Evidently, amino acids in the shell are slightly repulsive and again, dispersion energy is dominant. By extending the model from the "shell" (consisting of 338 atoms) to a larger section of protein (consisting of 722 atoms, see Figure 4) we obtained a slight increase in stabilisation energy (from 56 to 61 kcal mol^{-1}, an increase of about 10%). This data indicates that "second shell" amino acids contribute negligibly to the stabilisation of roscovitine.

Let us finally investigate the performance of the Cornell empirical potential. The sum of the 14 stabilisation energies (57.4 kcal mol^{-1}) is close to the MP2 value. We also found good agreement in the stabilisation energies for single clusters. Evidently, the Cornell empirical potential[19] is well suited for the present type of protein–ligand complexes and its use can be recommended. Upon extending the model, we obtain (as in the previous case) only a slight increase in stabilisation energy (from 57 to 62 kcal mol^{-1}, an increase of about 5%). The Cornell empirical potential is also feasible for molecular mechanics-Poisson–Boltzmann (generalised Born) solvation area (MM-PB(GB)SA) analysis of large protein–ligand or protein–protein complexes. Finally, the calculated values of interaction energies agree well with the interaction energies averaged over molecular dynamics (MD) simulations.[14]

Conclusions

1) The majority of stabilisation energy between roscovitine and protein originates from dispersion energy.
2) Due to the lack of dispersion energy, DFT methods fail to describe the roscovitine–protein interactions and their use cannot be recommended for inhibitor–protein studies. If the DFT energy is augmented empirically by London dispersion energy, reliable stabilisation energies can be achieved.
3) The Cornell empirical potential describes reasonable well the interaction between roscovitine and protein. This supports the use of this potential for future free-energy calculations.
4) A limited number of amino acid residues contribute significantly to the binding of roscovitine to cdk2 and, conversely, a rather large number of amino acids contribute negligibly. Mutation of the former, as well as the latter residues, can, thus, have a dramatic influence on the binding of roscovitine to the cavity and, consequently, also on the biological activity of roscovitine.
5) Besides providing alterations to an inhibitor, mutation of roscovitine will play a key role in the design of potential drugs. In subsequent work we will study both the changes in protein and the mutation of roscovitine. This will not be limited to interaction energy only. Although we believe (and have evidence) that the interaction energy contributes dominantly to a change of free energy of complexation, we will determine explicitly, besides interaction energies, the change of free energy of complexation of cdk2 with the ligand roscovitine.
6) The E81F82L83 (EFL) fragment with a dominant contribution to the interaction energy is conserved for cdk2, cdk1 and cdk3, which are highly homologous and have similar affinities to roscovitine. Cdk9 and cdk5 are both sensitive to roscovitine and have DFC and EFC motifs, respectively, instead of the EFL motif of cdk2. Cdk4 and cdk6, which are less sensitive to roscovitine, possess the EHV motif, in which phenylalanine is mutated to histidine. This finding reveals the important role of π–π interactions of the phenylalanine ring in roscovitine selectivity. In all cases, a change in the structure of roscovitine at the point of contact with these residues influences the strength of binding to cdk2. This fact is well documented by a reduction in the interaction of cdk with C8-substituted roscovitine analogues, due to disruption of the hydrogen bond between C8–H and the E81 carbonyl group.[15,26,27]

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