Crystal Structure of a Human Cyclin-Dependent Kinase 6 Complex with a Flavonol Inhibitor, Fisetin

Heshu Lu[1], Debbie Jimway Chang[2], Blandine Baratte[3], Laurent Meijer[3], and Ursula Schulze-Gahmen[1]

[1] Physical Bioscience Division at Lawrence Berkeley National Laboratory; [2] Department of Molecular Pharmacology, Stanford University;
[3] Station Biologique de Roscoff, C.N.R.S.Roscoff Cedex, Bretagne, France.

Abstract

Cyclin-dependent kinases (CDKs) play a central role in cell cycle control, apoptosis, transcription and neuronal functions. They are important targets for the design of drugs with antimitotic or antineurodegenerative effects. CDK4 and CDK6 form a subfamily among the CDKs in mammalian cells, as defined by sequence similarities. Compared to CDK2 and CDK5, structural information on CDK4 and CDK6 is sparse. We describe here the complex structure of human CDK6 in complex with a viral cyclin and a flavonol inhibitor, fisetin. Fisetin binds to the active form of CDK6, forming hydrogen bonds with the side chains of residues in the binding pocket that undergo large conformational changes during CDK activation by cyclin binding. The 4-keto group and the 3-hydroxyl group of fisetin are hydrogen bonded with the backbone in the hinge region between the N-terminal and C-terminal kinase domain, as has been observed for many CDK inhibitors. However, CDK2 and HCK kinase in complex with other flavonol inhibitors such as quercetine and flavopiridol showed a different binding mode with the inhibitor rotated by about 180°. The structural information of the CDK6-fisetin complex is correlated with the binding affinities of different flavonol inhibitors for CDK6. This complex structure is the first description of an inhibitor complex with a kinase from the CDK4/6 subfamily and can provide a basis for selecting and designing inhibitor compounds with higher affinities and specificities.

Overall Structure of the Complex

Figure 1. Molecular structure of fisetin, (3,7,3',4'-Tetrahydroxyflavone), C15H10O6, Mw 286.24.

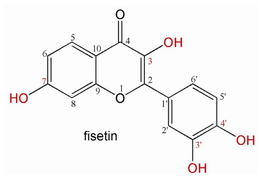


Figure 2. Schematic drawing of the CDK6-Vcyclin complex with bound fisetin inhibitor. CDK6 is shown in red with the PLSTIRE helix in purple, the T-loop in blue, and the hinge region in yellow. Cyclin is shown in green. Missing regions in the protein are labeled with stars and residue numbers at the chain interruptions. Fisetin is shown as a CPK model bound in the ATP binding pocket of the kinase.

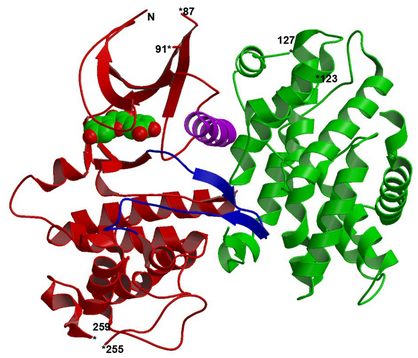


Table 1. Data Collection and Refinement Statistics

Data collection		Refinement			
Resolution range (Å)	50-2.9	Resolution (Å)	20-2.9	Ramachandran analysis (%)	
No. of observations	107,223	Data/parameter	0.78	Most favored	80.6
No. of unique reflections	12,984	R_{cryst}/R_{free}	0.260/0.313	Addition Allowed	17.3
Completeness (%)	91.4 (90.0)	No. of atoms	4173	Generously Allowed	2.1
Average *I/σ(I)*	18.6 (4.4)	CDK6	2241	Disallowed	0
R_{sym} (%)	11.7 (53.8)	Vcyclin	1911	Average B-factor (Å2)	58.8
Mosaicity	0.69	Fisetin	21	CDK6	65.8
		R.m.s. deviations		Vcyclin	50.9
		Bond (Å)	0.008	Fisetin	26.6
		Angles (°)	1.38		

Values in parentheses refer to the highest resolution shell.[1] $R_{cryst} = \sum h||Fobs(h)|-|Fc(h)||/\sum h|Fobs(h)|$ for all data, R_{free} was calculated from 7.5% of structure factor amplitudes that were excluded from refinement; [2] Overall/outer shell; [3] $R_{sym} = \sum hkl \sum i\ |I-<I>|/\sum hkl \sum I$; [4] Most favored region in Ramachandran plot as defined in PROCHECK.

- Fisetin (3,7,3',4'-Tetrahydroxyflavone) is a relatively small flavonol inhibitor with a hydroxyl-group in the 3 position of the benzopyran ring. This distinguishes it from several other flavonol inhibitors whose inhibitory activity on CDK6-Vcyclin was determined in this study as well (Figure 1& 5).
- The complex structure of CDK6-Vcyclin with the inhibitor fisetin is the first inhibitor complex structures with a kinase from the CDK subfamily consisting of CDK4 and CDK6 (Figure 2 & Table 1).
- Fisetin was found to bind in the ATP binding pocket which is located between the two kinase domains (Figure 2).

The Ligand-Binding Pocket with Bound Fisetin

Figure 3. Schematic drawings of fisetin interactions with residues in the CDK6 binding pocket. (**A**) Electron density for the bound inhibitor. The $a_{calc}(|Fo|-|Fc|)$ simulated annealing omit map was calculated to 2.9 Å resolution and contoured at 3 σ. (**B**) Difference electron density map for fisetin bound in the rotated orientation II. The map was contoured at 2.5 σ (purple) and -2.5 σ (green). The fisetin model in orientation I is shown in green, the fisetin model in orientation II in yellow. (**C**) Stereo view of fisetin interactions with residues in the CDK6 binding pocket. Hydrogen bonds are indicated as broken lines. (**D**) Schematic of CDK6 interactions with fisetin. Protein residues are shown as rectangular boxes labeled with the residue number and the total number of contacts in brackets. Protein side chain contacts are indicated by lines connecting the respective residue box and interactions to main chain atoms are shown as lines to the specific main chain atoms. Van der Waals contacts are indicated as broken lines, and H bonds by dashed lines.

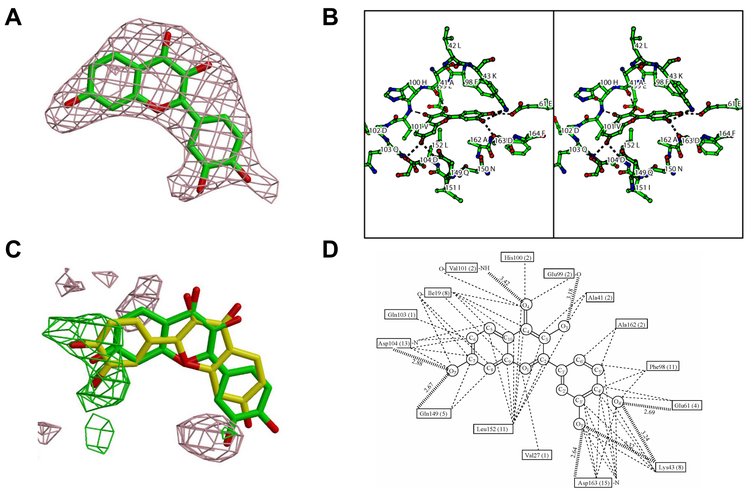
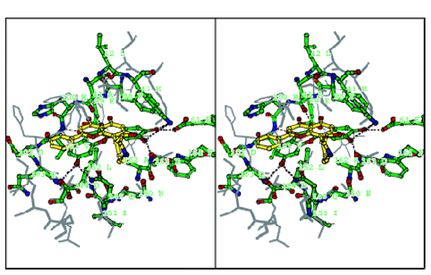


Table 2. Intermolecular contacts between CDK6 and fisetin[a]

CDK6 Residue (CDK2 number)	Total contacts (<4.11Å)	Hydrogen bond with fisetin atom
Ile19 (Ile10)	8	
Val27 (Val8)	1	
Ala41 (Ala31)	2	
Lys43 (Lys33)	8	1 with 3'OH, 1 with 4'OH
Glu61 (Glu51)	4	1 with 4'OH
Phe98 (Phe80)	11	
Glu99 (Glu 81)	2	1 with 3 OH
His100 (Phe82)	2	
Val101 (Leu83)	2	1 with 4 O
Gln103 (Gln85)	1	
Asp104 (Asp86)	13	1 with 7 OH
Glu149 (Gln131)	5	1 with 7 OH
Leu152 (Leu134)	11	
Ala162 (Ala144)	2	
Asp163 (Asp145)	15	1 with 3'OH
Total number	87	8

[a] Contacts are grouped into those with residues in the N-terminal domain (upper section), those with residues in the hinge region (middle section), and those with residues in the C-terminal domain (bottom section).

Figure 4. Comparison of the binding pockets of the CDK6-fisetin complex and the CDK2- deschloro-flavopiridol complex. The CDK6-fisetin complex is shown in green. Deschloro-flavopiridol is shown in yellow, and CDK2 in grey. The kinases were superimposed on the C-terminal kinase domains. Residue labels are shown for CDK6.



- The different combination of hydroxyl group may lead to different orientations of the bound inhibitors, as seen for flavopiridol versus fisetin complex.
- Fisetin may bind with higher affinity to the activated form of CDK6 than to the inactive apoenzyme.

Correlation of Structural Results with Binding Affinities

Figure 5. Molecular structure of six flavonol inhibitors of protein kinases, quercetin, apigenin, luteolin, kaempferol, chrysin and flavopiridol.

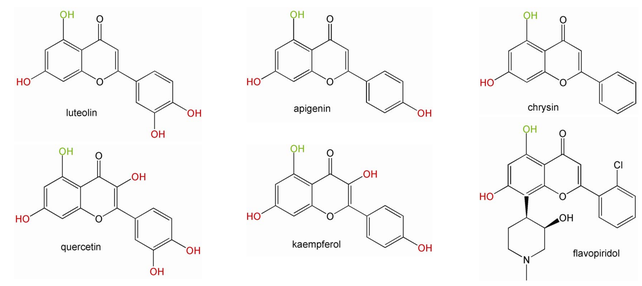
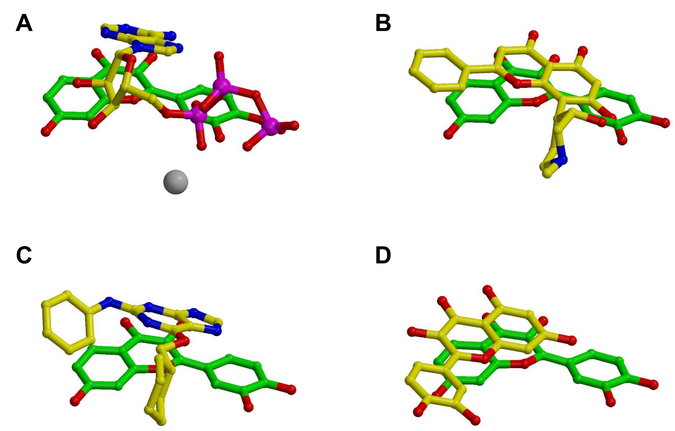


Table 3. Kinase Inhibition of Various Flavonoid Inhibitors[a]

	CDK6/cyclin	VCDK5/p25	CDK1/cyclinB	GSK-3[b]
Fisetin	0.85	0.57	0.79	0.42
Apigenin	1.7	1.6	4.0	1.4
Luteolin	>300	3.8	6.2	0.8
Quercetin	25	23	75	2.1
Chrysin	6	3.1	7.1	7.2
Kaempferol	22	51	41	3.5
Flavopiridol	0.08	0.17	0.21	0.28

[a] Enzyme activities were assayed as described in Materials and Methods, in the presence of increasing concentrations of inhibitor. IC50 values were calculated from the dose-response curves and are presented in micromolar; [b] Glycogen synthase kinase-3.

Figure 6. Comparison of the binding mode of various CDK ligands and inhibitors. All kinase complexes were superimposed on the C-terminal kinase domain using the program Overlap. Fisetin from the CDK6-fisetin complex is shown in green. Superimposed are four other ligands shown in yellow, (A) CDK2-ATP, (B) CDK2-deschloro-flavopiridol, (C) CDK2-NU6094, and (D) Hck-quercetin. The Mg atom coordinated to ATP in (A) is shown in grey.



- Additional substituent in the 2'-phenyl ring may create a inhibitor with higher affinity to CDK6.
- Exploring substitutions in the 6/7 –benzopyran ring may lead to a more specific inhibitor for CDK6.
- It's better to increase the structural information on CDK6-inhibitor complexes, to help to design more specific inhibitory compounds.

Acknowledgements

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