UDK 577.322, 577.152.271 **doi 10.15407/biotech7.06.029**

EFFECT OF PROTEIN KINASE CK2 INHIBITORS ON ITS CATALYTIC SUBUNITS CK2α AND CK2α′

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Received 04.06.2014

The effect of protein kinase CK2 inhibitors (with IC_{50} from 0.004 μM to 0.7 μM) from different chemical classes on the activity of CK2α and CK2α′ recombinant proteins have been studied. Biochemical tests shown that isozymes have different sensitivity toward same compounds. The most isoform-selective inhibitor was 4′-hydroxyflavone derivative (FLC26) with IC_{50} value 0.020 μM (CK2α) and 0.003 μM (CK2α′). To explain the difference between influence of FLC26 on the activities of two CK2 catalytic subunits their complexes with ATP-binding site of CK2α and CK2α′ were analyzed using molecular modeling techniques. The data obtained by molecular dynamics simulation (10 ns) has not provided a clear explanation of the difference between the inhibitory potency of the compound FLC26 towards the CK2 catalytic subunits. Thus, the reasons underlying the different activities of the same inhibitor on CKα and CKα′ require further investigations. An effective approach for this purpose would be X-ray analysis of complexes «compound FLC26-CKα/CKα′».

Key words: catalytic subunits of protein kinase CK2, docking, molecular dynamics simulation.

Protein kinase CK2 is a conserved Ser/Thr kinase that present in all eukaryotic cells. CK2 is highly pleiotropic and have many target-proteins (more than 300) which are present in most cell compartments. This enzyme is essential for cell viability and it takes part in such important processes as t-RNA and r-RNA synthesis, apoptosis, transformation, transcription, proliferation and control of cellular cycle [1]. The role of this protein kinase in the development of cancer, diabetes, viral, inflammatory and neurodegenerative diseases was determined [2].

Taking into consideration that CK2 regulates important physiological and pathological processes many studies have been directed towards determination of its three-dimensional structure. It was shown that CK2 is a tetramer consisting of two catalytic (α and/or α′) and two regulatory (β) subunits. Recently third catalytic subunit CK2α″ has been identified and characterized in human [3]. So, enzyme tetramer may be composed of ααββ, αα′ββ, α′α′ββ, αα″ββ, α′α″ββ, α″α″ββ.

Both subunits CK2α and CK2α′ have catalytic domains and are constitutively active whereas CK2β-subunits are regulatory and influence on the stability and substrate specificity of α-type subunits [4]. The global sequence similarity between catalytic subunits is about 75%, and this value increases to 86% if we consider the N-terminal fragment 1–329 (1–330 in CK2α′). On the contrary, the C-terminal domain is different in length (CK2α′ is 41 residues shorter then CK2α) and sequence similarity (only 38%) [5]. Other important feature of the isoenzymes structure is difference in the amino acid sequence of their ATP-binding sites hinge regions: CK2α-subunit contains His115-Val116 residues, and CK2α′ — Tyr116-Ile117. This feature can be used for development of isoform-specific inhibitors.

Although catalytic subunits are the products of the expression of different genes it was found that α-subunit is able to compensate the absence of CK2α′. Mice which have knock-out gene coding CK2α′ can birth viable offspring. It has to be noted that the ability of CK2α to compensate functionally for the lack of CK2α′ is not absolute because the male offspring was born sterile and displayed a defect in spermatogenesis while knock-out of the gene encoding CK2α-subunit led to

the animal death [6]. In experiments in vitro CK2α and CK2α′ showed similar enzymatic properties [7], so many researchers made no distinction between them. However, nowadays there are many evidences which prove the different localization of CK2 catalytic subunits in the organism, their various kinds of functions in the cell and their diverse role in the development of different diseases. For example, experiments with mice showed that CK2α is detected in almost all organs, whereas CK2α′ is found only in brain and testicles and had specific functions in these organs [8]. In adult chicken the highest level of CK2α was observed in spleen and heart tissue, the CK2α′-subunit — in liver, brain and ovaries [9]. In *Saccharomyces cerevisiae* the first isozyme (CKA1) is required for maintenance of cell polarity whereas the other isoform (CKA2) is involved in cell cycle progression. Additional confirmation of functional differences between CK2α and CK2α′ are isoform-specific interacting proteins the list of which is regularly updated [10].

Catalytic subunits have different levels of expression in various types of tumor. For example, D.W. Litchfield showed the difference between expression levels of CK2 catalytic subunits in metastatic tumors [11]. We have also carried out the theoretic analysis of data of gene expression of CK2 catalytic subunits in different types of tumor and cancer cell lines (SAGE analysis) using web resource (http://cgap.nci.nih.gov/SAGE). As a result of this study the following data have been obtained: the highest level of CK2α expression was observed in pancreatic and skin tumor cells; the highest level of CK2α′ expression was found in tumor cells of brain, breast and liver. In tumor cells of other organs there was no significant difference in the level of CK2 catalytic subunits.

Thus, the various functions and properties of CK2 individual molecular forms are the basis for development of isoform-specific inhibitors, which can be used for study and regulation of potentially pathogenic processes in cells.

In this paper it was determined the activity of known low molecular inhibitors [12–18] on the recombinant proteins of CK2 catalytic subunits and the attempt to explain the difference in activity of the same inhibitors towards the different subunits was made using molecular modeling techniques.

Materials and Methods

Production of recombinant protein of CK2α catalytic subunit

To amplify nucleotide sequence coding CK2α catalytic subunit the plasmid pCDNA4/TO containing insert of human CK2α cDNA clone (kindly provided by Dr. Ivan Gut, LICR London) and a pair of oligonucleotide primers were used. These primers (Forvard 5′- CAT GGA TCC CAT ATG TCG GGA CCC GTG CCA AGC AGG-3′ and Reverse 5′- CATA AAGCTTA CTG CTG AGC GCC AGC GGC AG -3′) have restriction sites of NdeI and HindIII. PCR-amplification was performed on thermocycler Perkin Elmer, Cetus using of native form of Pfu-polymerase (#EP0571, Fermentas, Lithuania) and dNTPs (#R0241, Fermentas, Lithuania) under the following conditions: at 94 °C for 5 min, 31 cycles (at 94 °C for 45 s, at 56 °C for 30 s, at 72 °C for 150 s), at 72 °C for 7 min. PCR-product (insert) after partial DNA reprecipitation (by Na-acetate-ethanol) was treated by NdeI (#FD0583, Fermentas, Lithuania) and HindIII (#FD0504, Fermentas, Lithuania) restriction endonucleases. The vector pET-28a was treated by the same restriction enzymes. The products of restriction were loaded onto the 0.8% agarose gel (TopVision LE GQ Agarose, #R0491, Fermentas, Lithuania) and electrophoresis was carried out in Tris-acetate buffer (50 mM Tris-acetate, pH 8.0, 20 mM Na-acetate, 2 mM EDTA) at 120 V. DNA of vector and insert was isolated from the gel using DNA isolation kit (QIAEX II Gel Extraction Kit, QIAGEN, Germany).

The purified fragments were ligated in ratio vector:insert as 1:5 during 3 hours at 23 °C. The cells of *E. coli* XL1 were transformed with products of ligation. The clones were selected directly on the solid nutrient medium LB with kanamycin. After transformation the plasmid was isolated from the clones by the alkaline lysis method [19].

The presence of CK2α insert in recombinant clones was confirmed by restriction with HindIII and NdeI and characteristic restriction with HincII (FD0494, Fermentas, Lithuania) and XhoI (FD0694, Fermentas, Lithuania), whose restriction sites are in the insert of CK2α cDNA.

Construct which was used for transformation had 20 additional N-terminal amino acids (MGSSHHHHHHSSGLVPRGSH), His-tag was among them (theoretical molecular weight of the recombinant protein was 47,306 кDa). The plasmid was isolated from

the clones that have fragments with the same size as target sequence. Competent cells of *E. coli* BL21 DE3 Rosetta were transformed by this plasmid and plated on the Petri dishes with the solid nutrient medium with selective antibiotics (kanamycin and chloramphenicol). Derived clones were cultivated at 30 °C on the liquid medium LB with 0.5 mM IPTG (isopropyl-β-D-thiogalactopyranoside) to induce protein expression. The recombinant protein was extracted from cell cultures by lysis and was analyzed in 10% PAGE by Laemmli method [20].

Producers of the recombinant protein were grown on the nutrient medium LB with selective antibiotics (kanamycin and chloramphenicol) up to optical density A_{600} = 0.5–1.0. After that the expression inducer IPTG was injected (final concentration was 0.5 mM) and incubation was continued for 16 hours at 30 °C. The cells were pelleted by centrifugation (10,000 g for 15 min at +4 °C) and resuspended in lysis buffer A (150 mM NaCl, 50 mM Tris, pH 7.5, 1 mM beta-mercaptoethanol) which amount was equal to 1/70 of the volume of cell culture and frozen. Then cells were thawed on ice and buffer A with lysozyme (final concentration 20 μg/ml) and PMSF (final concentration 2 mM) was added. After 20 min of incubation at +4 °C obtained suspension was exposed to ultrasonic treatment by disintegrator Labsonic (Sartorius, Germany). The fraction of soluble proteins with the target protein was isolated from the insoluble fraction by centrifugation (20,500 g for 20 min at +4 °C) and the concentration of total protein in the cell lysate was determined by Bradford [21].

Recombinant protein CK2α was purified by metal sepharose chromatography (TALON® Metal Affinity Resins, Clontech Laboratories, Inc., USA) using batch-method. Lysate was added to the sorbent (40 μl per 1 ml of the sorbent) and incubated for 2 hours at 4 °C under constant stirring (on the rotor). Sepharose with the bound protein was washed twice with buffer A and twice with buffer B for 5 min using inversion. The product was centrifuged between washes (3,000g for 45 s). The elution buffer composed of 300 mM NaCl, 350 mM imidazole, 50 mM Tris-HCl, pH 7.5.

To remove imidazole the obtained protein was dialyzed for 16 hours in the following buffer: 350 mM NaCl, 20 mM Tris-HCl, pH 7.5, 1 mM Na_2EDTA, 2 mM DTT and 0.1% Triton X-100. The amount of the purified protein was determined by Bradford, the purity was visually evaluated by electrophoresis in 10% PAGE.

Production of the recombinant protein of CK2α′ catalytic subunit

To amplify the sequence coding CK2α′ catalytic subunit (theoretical molecular weight of the recombinant protein was 43,749 кDa) the plasmid pCDNA4/TO containing the insert of human CK2α cDNA clone (kindly provided by Dr. Ivan Gut, LICR London) and the pair of oligonucleotide primers were used. These primers (Forvard -5′-CAT GGA TCC CAT ATG CCC GGC CCG GCC GCG GGC AG -3′ and Reverse 5′- CA TAA AGC TTA TCG TGC TGC CGT GAG ACC ACT GGA A -3′) have restriction sites of NdeI and HindIII. PCR-amplification was performed on thermocycler 2720 Thermal Cycler (Applied Biosystems, USA) using the native form of Pfu-polymerase and dNTPs under the following conditions: at 95 °C for 10 min, 31 cycles (at 94 °C for 40 s, at 55.5 °C for 35 s, at 71.5 °C for 120 s), at 72 °C for 7 min. DNA of vector and insert was isolated as it was described for CK2α catalytic subunit. Recombinant protein had 20 additional N-terminal amino acids (MGSSHHHHHHSSGLVPRGSH), His-tag was among them. The purified fragments were ligated in ratio vector:insert as 1:3 during 3 hours. The cells of *E. coli* XL1 were transformed with products of ligation. The plasmid was extracted from the clones obtained as it was described for CK2α catalytic subunit. The recombinant protein CK2α′ was isolated and purified the same way as CK2α.

Estimation of inhibition degree of human protein kinase CK2 catalytic subunits enzymatic activity

The kinase activity in the presence of synthetic compounds was determined using in vitro kinase assay (γ-^{32}P-ATP method). The volume of the reaction mixture was 20 μl and had the following composition: 3 μl 10^x buffer for CK2 (20 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM $MgCl_2$); 3 μl (6 μg) of peptide substrate RRRDDDSDDD (New England Biolabs); 0.02 μl of recombinant protein of CK2α catalytic subunit (approximately 20 ng of recombinant protein) or 0.035 μl of recombinant protein of CK2α′ catalytic subunit (approximately 7 ng of recombinant protein); 13 μl of distilled water. Aliquots of reaction mixture (19 μl per probe) were added to Eppendorf tube (volume 1.5 ml), then 1 μl of the inhibitor (previously solved in DMSO) in required concentration was added. To start the reaction the special mixture was added to each sample; this mixture had the following composition: 10 μl 150 μM ATP and 0.01 μl γ-[^{32}P]ATP with the activity

10 mCi/mM/ml (i.e.100 μCi per reaction). The final concentration of ATP in the reaction mixture was 50 μM. After incubation (for 25 min at 30 °C) the reaction was stopped by adding 8 μl of 5% solution of orthophosphoric acid; after that the reaction mixture was applied onto 20 mm phospocellulose disk paper (Whatman). Disks were washed three times with 0.75% solution of orthophosphoric acid and dried at room temperature. The level of radioactive signal was measured according to Cherenkov technique in a beta-counter (Rack Beta, Pharmacia). As the negative control 1 μl probe of DMSO (3.8%) was used. The degree of inhibition of CK2 catalytic subunits was defined as $-^{32}P$ incorporation ratio with or without inhibitor. The IC_{50} value was found by determination of the enzyme activity at different compound concentrations. To evaluate IC_{50} the concentration range of the compound (titration) was selected and coordinated by repeated experiments with the aim to plot the titration curve with upper and low asymptotes. The inhibitor was titrated at 10–16 concentration points which were obtained by two-fold dilution. Titration curves were plotted in the following coordinates: Y-axis — enzyme activity at each concentration of the inhibitors in CPS (counts per second); X-axis — logarithm of inhibitor concentration (lg[I]). For the mean value (the level of inhibitor concentration in the point which corresponds to the middle of the segment between upper and low asymptote) the inverse logarithm of inhibitor concentration was found, this calculated value is IC_{50}.

Molecular docking method

To obtain complexes «ligand-protein» we used DOCK 4.0 [22–25]. Molecular docking was carried out in ATP-binding sites of catalytic subunits of CK2 human protein kinase (PDB id: 1JWH and 3OFM) as previously described [16].

Molecular dynamics of complexes CK2-inhibitor in water environment

To study molecular dynamics simulation we used the crystal structures of catalytic subunits of CK2 protein kinase from Brookhaven Protein Data Bank (PDB id: 1JWH and 3OFM). Start position of the ligand in the active site of receptors was generated with DOCK software package. Molecular dynamics simulation was performed with GROMACS software package in the GROMOS96 force field [26]. Energy minimization of molecular complexes was carried out in explicit water environment with steepest descent energy minimization algorithm for 1,000 relaxation steps. At the next stage we calculated «limited» molecular dynamics. The period of water relaxation was 20 ps. The system «protein-ligand-water» was used for calculation of molecular dynamics simulation (10 ns).

Integration of the equations of motion was performed using the leapfrog algorithm [27], the initial velocity of atoms was randomly generated using Maxwell distribution. Molecular dynamics was evaluated at the temperature 300 K. To calculate electrostatic interactions we used algorithm Particle Mesh Ewald [28].

Results and Discussion

The study of inhibitory potency of compounds from different chemical classes toward catalytic subunits of protein kinase CK2 (CK2α and CK2α′) have been performed. These compounds belong to the classes of 4-aminothieno[2,3-d]pyrimidines [12], 3,4,5,6-tetrahydro-thiopyrano[2,3-d] thiazoles [13], 3-carboxy-4(1H)-quinolones [14], 4,5,6,7-tetrahalogeno-1H-isoindole-1,3(2H)-diones [15], 4′-hydroxyflavones [16], 4′-carboxyflavonoles [17], (thieno[2,3-d] pyrimidin-4-ylthio) carboxylic acids [18] and are selective ATP-competitive inhibitor with IC_{50} value in the range 0.004–0.9 μM.

Compounds test in vitro was carried out on the recombinant proteins CK2α and CK2α′. The process of their isolation and purification were described in «Materials and Methods». Purity of the derived proteins was confirmed by electrophoresis (Fig. 1, *A, B*). It has to be noted that in case of CK2α-subunit we revealed two protein bands instead of one. This degradation is due to protein cleavage by an 'autoproteolytic' process during purification. It is well-known feature of recombinant CK2α which is purified in heterologous systems by one-step chromatography. The reason for this cleavage of recombinant protein CK2α into two parts were throughly studied and described in [29, 30]. The electrophoregram of recombinant protein CK2α′ is shown at Fig. 1, *B*; the similar results were described in [7].

The results of biochemical tests in vitro are given in the Table.

Subunit CK2α was more sensitive towards the compounds from the classes of 3-carboxy-4(1H)-quinolones (compound 7), 4,5,6,7-tetrahalogeno-1H-isoindole-1,3(2H)-diones (compounds 43 and 46) and (thieno[2,3-d]pyrimidin-4-ylthio) carboxylic acids (compounds 5p, 6a, 6b and 6d). The activity of CK2α′ was more efficiently inhibited by the compounds from the classes of 3,4,5,6-tetrahydro-thiopyrano[2,3-d] thiazoles (compounds TTT1 and TTT7) and

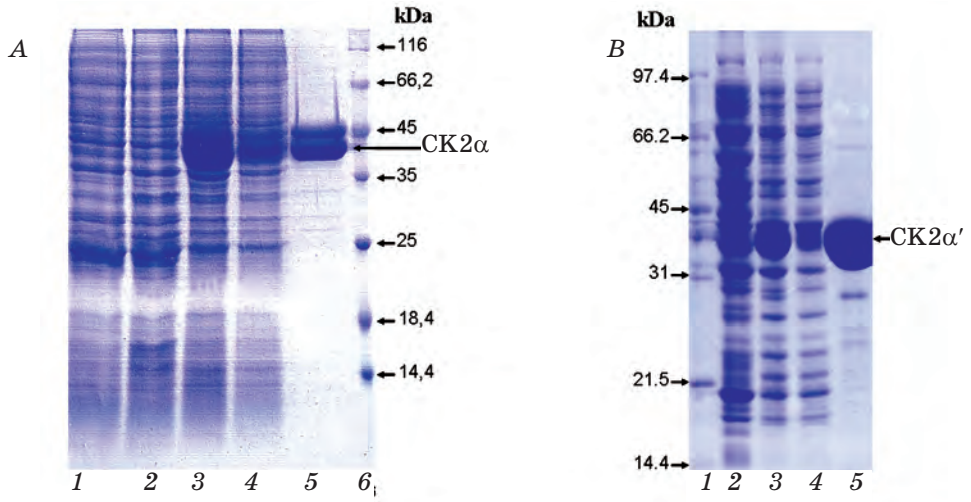


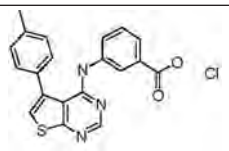
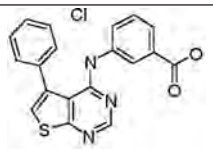
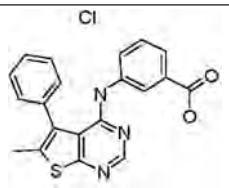
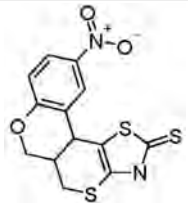
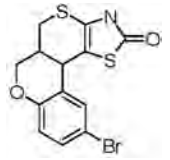
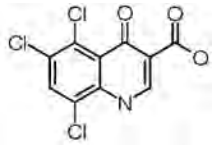
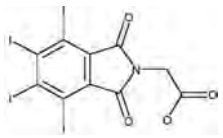
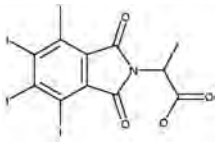
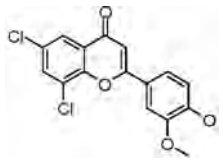
Fig. 1. **The electrophoregram of recombinant proteins:**
A — CK2α: *1* — protein fraction after cell lysis, the fraction contains target-product;
2, 3 — protein lysates of *E. coli* BL21 DE3 Rosetta cells, which were cultivated on LB medium without IPTG and with it, respectively;
4 — gap; *5* — recombinant protein after purification; *6* — marker.
B — CK2α′: *1* — marker;
2, 3 — protein lysates of *E. coli* BL21 DE3 Rosetta cells, which were cultivated on LB medium without IPTG and with it, respectively;
4 — gap; *5* — recombinant protein after purification.
Hereinafter the results of typical experiments were shown.

4′-carboxyflavonoles (compounds FLC21 and FLC26). For 4-aminothieno[2,3-d]pyrimidines (compounds NHTP23, NHTP25 and NHTP33) and 4′-hydroxyflavones (compounds FNH68 and FNH79) the situation was ambiguous: some compounds had stronger inhibitory properties toward CK2α, whereas the other — toward CK2α′. On the whole, the inhibitory potency (value IC_{50}) of the inhibitors of CK2α and CK2α′ subunits was differed in 1.6–6.7 times, flavone derivatives had pronounced difference. The most isoform-selective compound was 4-(6,8-dibromo-3-hydroxy-4-oxo-4H-chromen-2-il) benzoic acid (FLC26). This inhibitor suppressed CK2α′ activity more than 6 times compare with CK2α (IC_{50} for CK2α = 0.02 μM and for CK2α′ = 0.003 μM). It was interesting that compound FNH79 which has the chemical structure similar to FLC26 had stronger inhibitory potency against CK2α catalytic subunit.

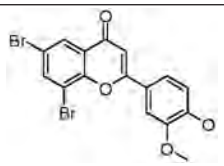
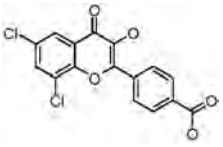
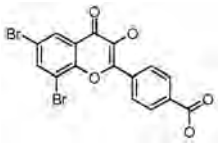
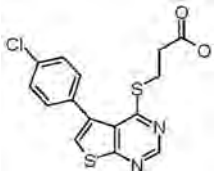
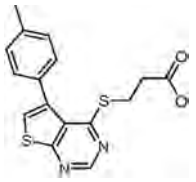
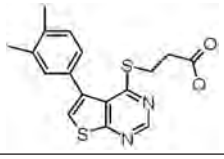
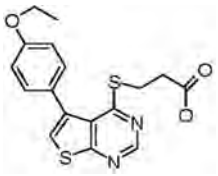
To explain the different activity of the same inhibitors towards two isoforms CK2 the «compound FLC26–CK2α/CK2α′» complexes have been obtained with the molecular docking method. As mentioned before ATP-binding sites of human catalytic subunits CK2 differ a few residues (CK2α′ has Tyr116–Ile117; CK2α has His115–Val116) [31]. These amino acids are localized in the hinge region, which is important for the formation of ligand-receptor interaction [32].

After analysis of obtained «ligand–CK2α/CK2α′» complexes (Fig. 2, *A*, *B*) it was established that the difference in amino acid residues of ATP-acceptor sites of the catalytic subunits had no effect on the location of studied ligands. All inhibitors were complementary to ATP-binding pockets of CK2α and CK2α′ and formed some hydrophobic contacts and hydrogen bonds. For example, compound FLC26 formed hydrophobic interactions with amino acids Leu45, Val66, Ile95, Phe113, Val116, Met163 and Ile174 in the CK2α active site. Besides them this inhibitor formed four intermolecular hydrogen bonds. The first one was between oxygen atom in the fourth position of heterocycle and nitrogen atom of the main chain of Val116; the second one was between 3-hydroxyl group and the main chain of Glu114. The third and the fourth hydrogen bonds were formed due to the ligand carboxyl group which was oriented towards the hydrophobic site 1, namely between oxygen atoms of carboxylic acid residue and nitrogen atoms of the side chain of Lys68 and main chain of Asp175. In the active site of CK2α′ the compound FLC26 was fixed due to hydrophobic contacts with the same amino acid residues (Leu46, Val67, Ile96, Phe114, Ile117, Met164, Ile175). Hydrogen bonds were formed between Ile117, Glu115, Lys69, Asp176 and the same ligand atoms as in the case of the «ligand–CK2α» complex.

The structure of the compounds and *in vitro* data (IC_{50}, μM) of their inhibitory activity towards protein kinase CK2 holoenzyme and its catalytic subunits

№ of the compounds	Chemical structure	IC_{50}, μM		
		CK2 ($\alpha\alpha\beta\beta$), μM	CK2α, μM	CK2α', μM
NHTP23		0.01 [12]	0.126	0.025
NHTP25		0.065 [12]	0.04	0.2
NHTP33		0.008 [12]	0.02	0.005
TTT1		0.7 [13]	3.3	1.48
TTT7		0.15 [13]	1.4	0.4
7		0.3 [14]	0.25	0.52
43		0.3 [15]	0.12	0.45
46		0.15 [15]	0.1	0.16
FNH68		0.01 [16]	0.005	0.003

Table

FNH79		0.004 [16]	0.003	0.006
FLC21		0.04 [17]	0.016	0.003
FLC26		0.009 [17]	0.02	0.003
5p		0.175 [18]	0.08	0.2
6a		0.1 [18]	0.16	0.32
6b		0.175 [18]	0.07	0.25
6d		0.125 [18]	0.11	0.63

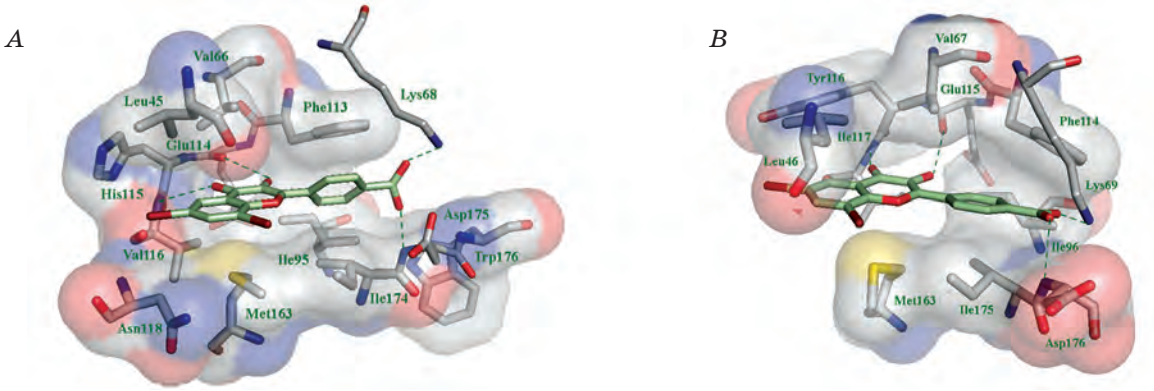


Fig. 2. **Binding mode of compound FLC26 in the ATP-binding site of catalytic subunits CK2α (*A*) and CK2α′ (*B*) obtained with molecular docking method. Intermolecular hydrogen bonds are shown by the dotted lines**

To study why FLC26 had different inhibitory activity towards CK2α and CK2α' the analysis of complexes of this compound with CK2 catalytic subunits was carried out with molecular dynamics simulation. This method shows the presence of hydrogen bonds more accurately and evaluates their stability over time. The molecular dynamics simulation of «compound FLC26–CK2α/CK2α'» complexes was performed in explicit water environment during 10 ns.

The RMSD plot (Fig. 3) for this ligand demonstrates that the both complexes were stable, the tendency to dissociation wasn't observed.

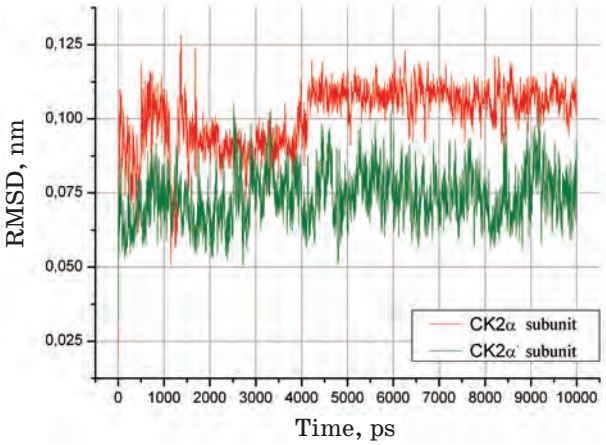


Fig. 3. **The root-mean-square deviation (RMSD) of compound FLC26 in the complex with CK2α (red colour) and CK2α′ (green colour) subunits during molecular dynamics simulation (smoothed by Savitzky-Golay method, 13 steps)**

The presence of nine hydrogen bonds formed between compound FLC26 and CK2α during molecular dynamics simulation was shown at Fig. 4, *A*. The bonds with Val116, Lys68 and Asp175 were stable. The bonds between oxygen #4 and main chain of Glu114 and between oxygen #3 and side chain of Ser51 periodically occurred for a few ps.

The hydrogen-bond existence map (Fig. 4, *B*) obtained from molecular dynamics simulation of the «compound FLC26-CK2α′» complex was something different. Initially the hydrogen bonds were formed between the oxygen atom #5 and the main chain of Asp176 and the side chain of Lys69. However, from 4,100 ps the carboxyl group of ligand rotated and formed two new hydrogen bonds [O3 — Asp176(N) and O3 — Lys69(N)]. These bonds were stable until the end of molecular dynamics. The hydrogen bond between the nitrogen atom of Ile117 with oxygen atom of chromenone was stable all time. The bonds between the ligand and Glu115 and Ser52 were same to ones with Glu114 and Ser51 in the «compound FLC26–CK2α» complex.

The described hydrogen bond networks (Fig. 4, *A, B*) correlates well with the curves of the sum of Culomb and Lennard-Jones interaction energies (Fig. 5, *A, B*). Significant slump of the energy curve of interaction of compounds FLC26 with CKα and CKα′ subunits (to −375 Kj/mol) during molecular dynamics simulation is associated with emergence and/or disruption of hydrogen bonds between oxygen of carboxyl group of ligand and Ser51 (CKα) and Ser52 (CKα′) amino acids residues, respectively. Obtained data suggest an important role of these intermolecular hydrogen bonds in the «compound FLC26–CKα/CKα′» complexes.

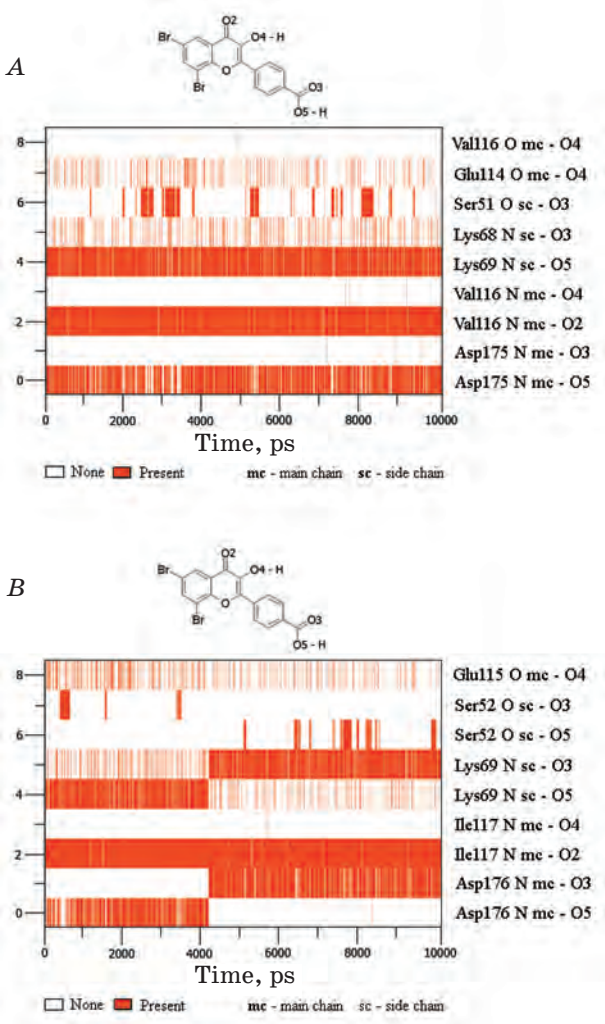


Fig. 4. **Hydrogen-bond existence map of complexes «compound FLC26-CKα» (*A*) and «compound FLC26-CKα′» (*B*) during a 10 ns molecular dynamics simulation**

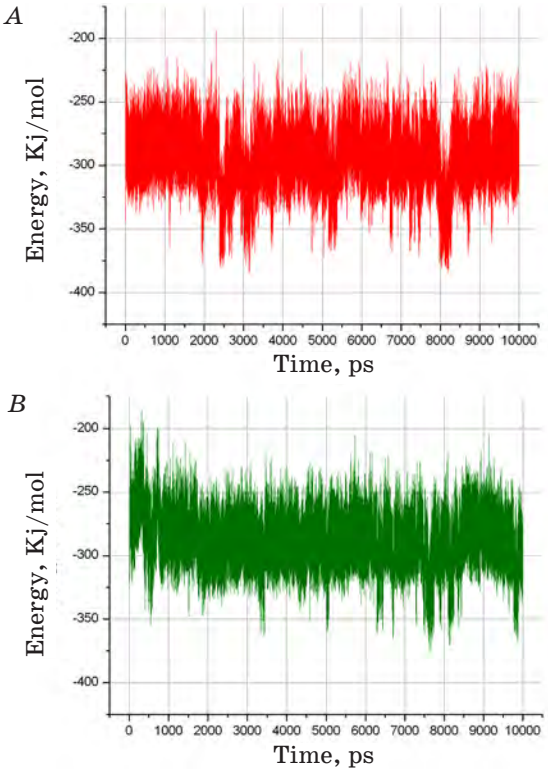


Fig. 5. **Sum of Coulomb and Lennard-Jones interaction energies for complexes «compound FLC26-CKα» (*A*) and «compound FLC26-CKα′» (*B*) during a 10 ns molecular dynamics simulation (smoothed by Savitzky-Golay method, 13 steps)**

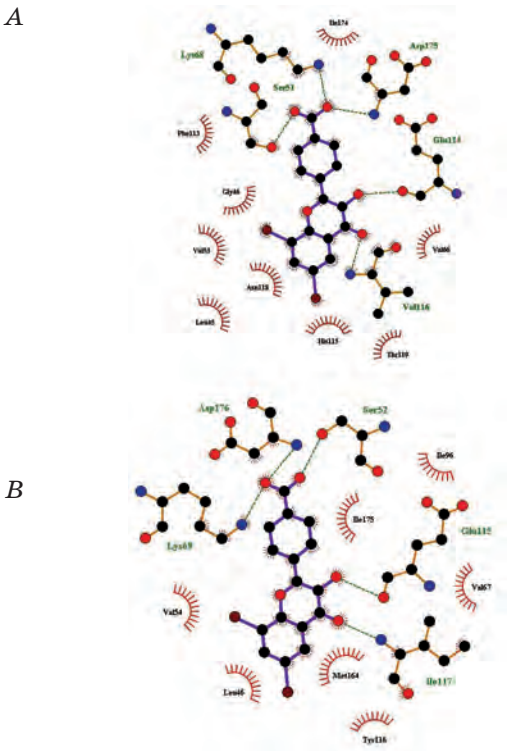


Fig. 6. **Binding mode of compound FLC26 in the ATP-binding site of catalytic subunits CK2α (*A*) and CK2α′ (*B*) obtained with molecular dynamics simulation. Intermolecular hydrogen bonds are shown by the dotted lines**

The binding mode calculated with molecular dynamics simulation (Fig. 6, *A*, *B*) had slight difference compared to the one obtained with docking (Fig. 2, *A*, *B*). The thirst is that the docking determined no hydrogen bonds with Ser51 and Ser52 for «compounds FLC26-catalytic subunits» complexes. The second is that the data obtained during molecular dynamics simulation shown that the hydrogen bonds with Lys68/69 and Asp175/176 amino acids residues were formed by two different oxygen atoms of carboxyl group, not one.

Thus, the ability of known inhibitors of protein kinase CK2 to selectively inhibit the activity of two isoforms of the catalytic subunit have been studied. It was shown that the difference in the activity of the same compounds toward CKα and CKα′ is ranged from 1.6 to 6.7 times. Analysis of «inhibitor-CKα/CKα′» complexes obtained by molecular modeling technique didn't explain appearance of such difference. This issue requires further investigation using crystallographic analysis methods.

This work was supported by the National Academy of Sciences of Ukraine (Grant 0112U004110).

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ВПЛИВ ІНГІБІТОРІВ ПРОТЕЇНКІНАЗИ CK2 НА ЇЇ КАТАЛІТИЧНІ СУБОДИНИЦІ CK2α І CK2α′

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Метою роботи було дослідження впливу 19 інгібіторів протеїнкінази CK2 (з IC_{50} від 0,004 мкМ до 0,7 мкМ) різних хімічних класів на активність рекомбінантних протеїнів CK2α та CK2α′. Біохімічні тести показали, що ізозими мають різну чутливість стосовно цих сполук. Найбільш ізоформно-селективним інгібітором виявилося похідне 4′-гідроксифлавону (FLC26) з IC_{50} = 0,020 мкМ (CK2α) та 0,003 мкМ (CK2α′). Для пояснення різниці впливу FLC26 на активність каталітичних субодиниць CK2 було проаналізовано його комплекси з АТФ-зв'язувальною кишенею CK2α та CK2α′ за допомогою методів молекулярного моделювання. Дані, одержані в результаті моделювання молекулярної динаміки комплексів протягом 10 нс не дали чіткого пояснення виникненню різниці між інгібіторною активністю сполуки FLC26 на каталітичних субодиницях CK2. Причини, які лежать в основі вияву різної активності одного й того самого інгібітора на CKα та CKα′ потребують подальших досліджень. Ефективним підходом для цього може бути рентгеноструктурний аналіз комплексів «сполука FLC26–CKα/CKα′»

Ключові слова: каталітичні субодиниці протеїнкінази CK2, докінг, молекулярна динаміка.

ВЛИЯНИЕ ИНГИБИТОРОВ ПРОТЕИНКИНАЗЫ CK2 НА ЕЕ КАТАЛИТИЧЕСКИЕ СУБЪЕДИНИЦЫ CK2α И CK2α′

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Целью работы было исследование влияния ингибиторов протеинкиназы CK2 (с IC_{50} от 0,004 мкМ до 0,7 мкМ) различных химических классов на активность рекомбинантных протеинов CK2α и CK2α′. Биохимические тесты показали, что изозимы имеют разную чувствительность по отношению к этим соединениям. Наиболее изоформно-селективным ингибитором оказалось производное 4′-гидроксифлавона (FLC26) с IC_{50} = 0,020 мкМ (CK2α) и 0,003 мкМ (CK2α′). Для объяснения разницы влияния FLC26 на активность каталитических субъединиц CK2 были проанализированы его комплексы с АТФ-связующим карманом CK2α и CK2α′ с помощью методов молекулярного моделирования. Данные, полученные в результате моделирования молекулярной динамики комплексов в течение 10 нс, не дали четкого объяснения возникновению разницы между ингибиторной активностью соединения FLC26 на каталитических субъединицах CK2. Причины, лежащие в основе проявления различной активности одного и того же ингибитора на CKα и CKα′, требуют дальнейших исследований. Эффективным подходом для этого может быть рентгеноструктурный анализ комплексов «соединение FLC26–CKα/CKα′».

Ключевые слова: каталитические субъединицы протеинкиназы CK2, докинг, молекулярная динамика.