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MOLECULAR DYNAMICS STUDIES OF COMPLEXES OF (\pm)-GOSSYPOL WITH CDK2 AND SARS-COV-2 MPRO PROTEINS

Annotation

This study presents molecular docking and molecular dynamics (MD) analyses of the interactions between (\pm)-gossypol (Gp) and two biologically important target proteins: the SARS-CoV-2 main protease (Mpro) and cyclin-dependent kinase 2 (CDK2). The molecular docking results showed that both isomers occupied similar binding regions and were therefore selected for MD simulations. The MD results demonstrated that PGp and MGp formed stable complexes with targets, exhibiting comparable conformational stability, binding modes, and trajectory convergence throughout the simulations. In addition, MM(PB/GB)SA free-energy calculations indicated that PGp and MGp possess similar binding affinities toward both Mpro and CDK2.

Keywords: (\pm)-Gossypol, Atropoisomers, Main protease of SARS COV2, Cyclin-dependent kinase 2, Molecular docking, Molecular dynamics.

МОЛЕКУЛЯРНО-ДИНАМИЧЕСКИЕ ИССЛЕДОВАНИЯ КОМПЛЕКСОВ (\pm)-ГОССИПОЛА С БЕЛКАМИ CDK2 И SARS-COV-2 MPRO

Аннотация

В данном исследовании представлены результаты анализа молекулярной динамики (МД) взаимодействий между (\pm)-госсиполом (Gp) и двумя биологически важными целевыми белками: основной протеазой SARS-CoV-2 (Mpro) и циклин-зависимой киназой 2 (CDK2). Результаты молекулярного докинга показали, что оба изомера занимают схожие области связывания и поэтому были выбраны для моделирования МД. Результаты МД продемонстрировали, что PGp и MGp образуют стабильные комплексы с целевыми белками, демонстрируя сопоставимую конформационную стабильность, режимы связывания и сходимость траектории на протяжении всего моделирования. Кроме того, расчеты свободной энергии MM(PB/GB)SA показали, что PGp и MGp обладают схожей аффинностью связывания как с Mpro, так и с CDK2.

Ключевые слова: (\pm)-Госсипол, Атропизомеры, Основная протеаза SARS-CoV-2, Циклин-зависимая киназа 2, Молекулярный докинг, Молекулярная динамика.

(\pm)-GOSSYPOLNING CDK2 VA SARS-COV-2 MPRO OQSILLARI BILAN KOMPLEKSLARINING MOLEKULAR DYNAMIK TADQIQOTLARI

Annotation

Ushbu tadqiqotda (\pm)-gossipol (Gp) va ikkita biologik muhim nishon oqsillar: SARS-CoV-2 asosiy proteazasi (Mpro) va siklinga bog'liq kinaza 2 (CDK2) o'rtasidagi o'zaro ta'sirlar molekulyar doking va molekulyar dinamika usullarida o'rganildi. Molekulyar doking orqali ikkala izomerining ham qariyb bir xil saytga bog'langan komplekslari MD simulyatsiyalari uchun tanlandi. MD natijalari PGp va MGp nishon oqsillari bilan barqaror komplekslarni hosil qilganligi simulyatsiyalar davomida aniqlandi. Bundan tashqari, MM(PB/GB)SA erkin energiya hisoblashlari ikkala izomerining ham nishon oqsillariga bog'lanish energiyalari deyarli bir xil ekanligini ko'rsatdi.

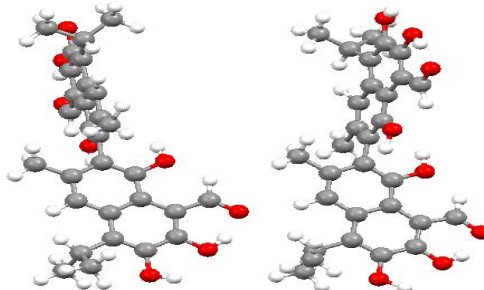
Kalit so'zlar: (\pm)-Gossipol, Atropoizomerlar, SARS-COV-2 Mpro, Siklinga bog'liq kinaza 2, Molekulyar doking, Molekulyar dinamika.

Kirish. Gossipol paxta chigitidan ajratib olingan tabiiy polifenol dialdegid bo'lib, u tautomer va stereoisomer (atropizomer) shakllari tufayli eksperimental va nazariy tadqiqotlarda chuqur o'rganilgan [1-3]. Gossipol molekulasining saratonga, viruslarga qarshi faol ekanligi aniqlangan [4-7]. Bu molekullarning ajoyib xususiyatlaridan biri, uning erituvchilar va kichik organik birikmalar bilan "mehmon" va "mezbon" tipidagi supramolekulyar komplekslar hosil qilishidir [8].

Mavzuga oid adabiyotlar tahlili. Stereoizomeriya natijasida gossipol (+)-gossipol (PGp) va (-)-gossipol (MGp) enantiomer shakllarda mavjud bo'ladi [9-11]. Bu izomerlarning farmakologik va toksikologik xususiyatlarida farq mavjudligi aniqlangan. Ya'ni, MGp *in vitro* tadqiqotlarida (+)-analogiga (PGp) nisbatan kuchliroq saratonga qarshi ta'sir ko'rsatadi [4]. Hozirga qadar nazariy hisoblashlarda (-)-gossipolning (+)-gossipolga nisbatan faolroq ekanligi aniqlanmagan. Umuman olganda molekulyar doking, molekulyar dinamika simulyatsiyalari va MMGBSA tahlili [12] yordamida (-)-gossipolning (+)-gossipolga nisbatan yuqori faollik ko'rsatishini baholash mumkinmi? Ko'pchilik molekulyar doking dasturlarida izomerlar orasida o'zaro

o'tishlar kuzatilishi [13] sababli bu savolga javobni keng ko'lamdagi molekulyar dinamik (MD) tadqiqotlar natijasida topish mumkin. Shuni inobatga olgan holda, ushbu ishda nishon oqsillari faol markazida deyarli bir xil saytda joylashgan MGp va PGp molekulalarining (1-rasm) CDK2 va SARS-COV-2 Mpro oqsillari bilan hosil qilgan komplekslari MD tadqiqotlari amalga oshirildi.

Ma'lumki, siklinga bog'liq kinaza 2 (CDK-2) ko'krak va tuxumdon saratoni kabi bir qancha xavfli o'smalarni davolashda asosiy terapevtik nishon bo'lib, saraton kasalligini davolashda dori qarshiligini yengish uchun juda muhimdir [14]. Shu sababli, CDK2 oqsiliga yaxshiroq bog'lanadigan birikmalar saratonni davolashda samaraliroq bo'lishi mumkin deb qaraladi. Gossipolning viruslarga qarshi faolligi mavjudligi sababli SARS-CoV-2 asosiy proteazasi (Mpro) ham nishon oqsili sifatida tanlandi [4-7].



1-Rasm. PGp (chapda) va MGp (o'ngda) atropoizomerlarning fazoviy tuzilishi

Tadqiqot metodologiyasi. Oqsillarning uch o'lchamli geometriyasini ifodalovchi fayllari (pdb fayl) oqsil ma'lumotlari bazasidan (Protein Data Bank-PDB, <https://www.rcsb.org/>) yuklab olindi. 1B38 va 6LU7 oqsillari mos ravishda CDK-2 va SARS-COV-2 M^{pro} oqsillari sifatida tanlandi. Tanlangan oqsillar Biovia Discovery Studio Visualizer v21.1 (Dassault Systemes BIOVIA, USA) dasturida suv va ligand molekulalaridan tozalandi. Oqsillarning faol markazlari CB-Dock2 [15] serverida aniqlandi.

MGp [9] va PGp [3] izomerlarining molekulyar va kristallografik ma'lumotlari Kembrij kristallografik ma'lumotlar bazasidan (CCDC) olindi. Nishon oqsillari va enantiomerlar AutoDock Tools [16] dasturida pdbqt fayllariga o'girildi. Molekulyar doking tadqiqotlari AutoDock Vina [17] dasturida amalga oshirildi.

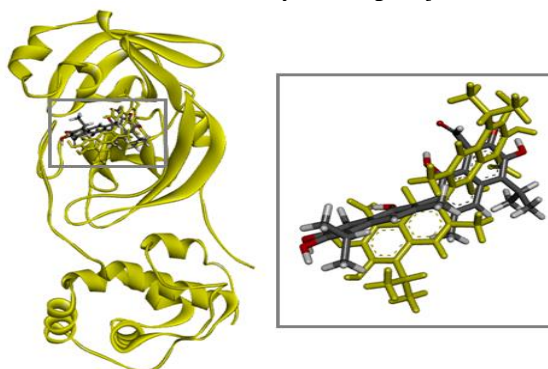
Tanlangan komplekslarning molekulyar dinamika (MD) simulyatsiyalari GROMACS 2025.3 dasturiy paketida [18] AMBER99SB-ILDN [19] kuch maydoni qo'llangan holda amalga oshirildi. MGp va PGp molekulalari uchun topologiya va geometrik parametr fayllari ACPYPE [20] serveri yordamida, AM1-BCC zaryad taqsimoti asosida generatsiya qilindi.

Dastlab, komplekslar davriy chegara shartlari ostida dodekaedr qutiga joylashtirildi. Tizim TIP3P suv modeli yordamida solvatatsiya qilinib, fiziologik sharoitni modellashtirish maqsadida Na⁺ va Cl⁻ ionlari qo'shib, umumiy ion konsentratsiyasi 0.15 M ga keltirildi. Shundan so'ng hosil bo'lgan kompleks-suv-ion tizimi energiya minimallashtirish bosqichidan o'tkazildi.

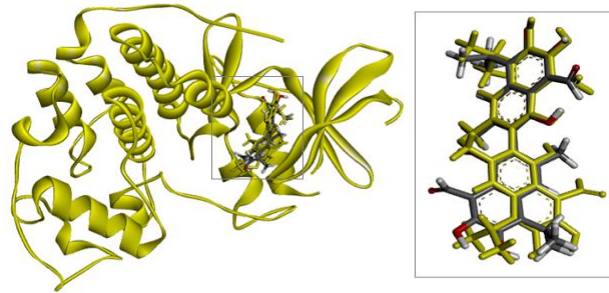
Keyingi bosqichda tizim avval doimiy hajm va harorat sharoitida (NVT ansambl), so'ngra doimiy bosim va harorat sharoitida (NPT ansambl) muvozanat holatiga keltirildi. Harorat va bosimni barqaror ushlab turish uchun mos termostat va barostat algoritmlaridan foydalanildi. Muvozanatlash bosqichlaridan so'ng, asosiy bosqich (production phase) sifatida 200 nanosekund (ns) davomida molekulyar dinamika simulyatsiyalari amalga oshirildi.

Bajarilgan 200 ns MD simulyatsiya natijasida olingan trayektoriya fayli 2000 ta vaqt kadrlari (frame) dan iborat bo'lib, ushbu trayektoriyaning oxirgi 10 ta kadriga asosan MM(PB/GB)SA [12] usuli yordamida oqsil-ligand kompleksi uchun bog'lanish energiyalari hisoblandi. Molekulyar dinamika simulyatsiyalari natijalari tizim barqarorligi va dinamik xususiyatlari o'rtacha kvadratik og'ish (RMSD, nm) parametri orqali baholandi.

Tahlil va natijalar. MGp va PGp molekulalarining 6LU7 (2-rasm) va 1B38 (3-rasm) oqsillari faol markazlarining deyarli bir xil saytida joylashgan komplekslari AutoDock Vina molekulyar doking natijalari asosida tanlab olindi.



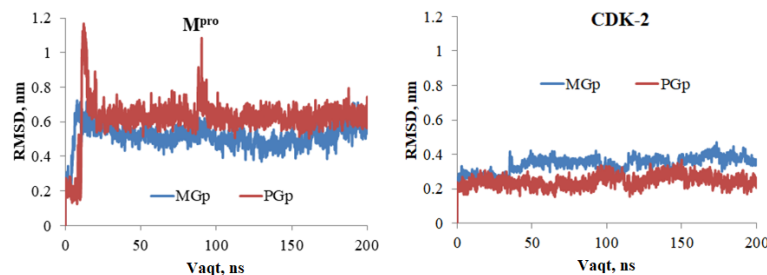
2-Rasm. MGp-6LU7 (sariq rangda) va PGp-6LU7 komplekslarining taqqoslanishi



3-Rasm. MGp-1B38 (sariq rangda) va PGp-1B38 komplekslarining taqqoslanishi

MD simulyatsiya (200 ns) orqali molekulyar tizimlarning dinamik hatti-harakatlarini tahlil qilish uchun RMSD grafiklari hosil qilindi. Mpro (6LU7) va CDK-2 (1B38) bilan MGp/PGp komplekslari uchun RMSD grafigi 4-rasmda keltirilgan. MGp-6LU7 kompleksi uchun dastlabki fazada (0-20 ns) va yana 100 ns atrofida nisbatan sezilarli tebranishlar kuzatildi. Undan keyin tebranishlar kamayib, RMSD 0.6-0.7 nm atrofida barqarorlashdi, bunda tizimning muvozanat holatiga kelganligi kuzatildi. PGp-6LU7 kompleksi holatida esa RMSD dastlab taxminan 0.2 nm dan 0.7 nm gacha ko'tarildi, keyin asta-sekin pasayib, taxminan 0.6 nm gacha kamaydi.

MD simulyatsiyasi davomida MGp-1B38 va PGp-1B38 komplekslari RMSD diagrammasida mos ravishda 0.3–0.4 nm va 0.2–0.3 nm oraliqdagi kichik tebranishlar kuzatiladi. Bu komplekslarda ham tizim muvozanat holatiga erishgan.



4-Rasm. MGp va PGp izomerlarining 6LU7 (Mpro) va 1B38 (CDK-2) oqsillari bilan hosil qilgan komplekslari MD simulyatsiya natijalarining o'rtacha kvadratik og'ish (RMSD) kattaligi bilan baholanishi

gmxMMGBSA dasturi [12] oqsil-ligand o'zaro ta'sirining bog'lanish erkin energiyalarini Puasson-Boltsman tenglamasini (PB, MMPBSA usuli) yechish yoki umumlashtirilgan Born usuli (GB, MMGBSA usuli) yordamida baholaydi. PB usuli GB usuliga nisbatan oqsil-ligand o'zaro ta'sirining yanada keng qamrovli va aniq tavsifini beradi [12, 53]. MGp va PGp ning Mpro va CDK-2 oqsillariga bog'lanish erkin energiyalari ikkala usul yordamida ham baholandi. O'rtacha bog'lanish energiyalari, mos keladigan entalpiya (ΔH) va entropiya (ΔS) o'zgarishlari bilan birga 1-jadvalda keltirildi. Bog'lanish erkin energiyasi ($\Delta G_{\text{bind}} = \Delta H - T\Delta S$) ushbu parametrlar asosida hisoblab chiqildi.

MMGBSA usuliga ko'ra, MGp PGp izomeriga nisbatan Mpro SARS-COV-2 oqsiliga nisbatan kuchliroq bog'lanishini namoyon etdi, bog'lanish energiyalari mos ravishda -30.10 kkal/mol va -27.13 kkal/mol ni tashkil qildi (1-jadval). Ammo MMPBSA natijalari PGp (-23.02 kkal/mol) ning MGp (-20.48 kkal/mol) ga nisbatan biroz ustunligini ko'rsatdi. Shunga o'xshash tendentsiya MGp va PGp ning 1B38 oqsili (CDK2) bilan bog'lanish energiyalari holatida ham kuzatildi, bu yerda PGp MMGBSA usuliga ko'ra MGp ga nisbatan biroz ustunlikka ega. Ammo, MMGBSA ma'lumotlariga ko'ra, MGp PGp ga nisbatan biroz ustunlikka ega (1-jadval).

Mpro-MGp kompleksining barqarorlashishiga MET49, ASN142, GLY143, MET165, PRO168, GLN189 va THR190 aminokislota qoldiqlari hissa qo'shishi aniqlandi. Mpro-PGp kompleksi barqarorlashishi uchun esa HIS41, MET165, PRO168, ALA191, GLN192 aminokislota qoldiqlari hissa qo'shishi aniqlandi.

1-Jadval. MMGBSA va MMPBSA usullari yordamida PGp va MGp ning SARS-COV-2 Mpro va 1B38 oqsillari bilan o'zaro ta'siri asosida aniqlangan ΔH , $T\Delta S$ va ΔG_{bind} kattaliklarning o'rtacha qiymatlari (kkal/mol)

Kattalik	MMGBSA	MMPBSA
	M ^{pro} – Musbat Gossipol	
ΔH	-31.88± 2.33	-27.76 ± 2.42
-TAS	4.74 ± 3.09	4.74 ± 3.09
ΔG_{bind}	-27.13 ± 3.87	-23.02 ± 3.92
	M ^{pro} – Manfiy Gossipol	
ΔH	-35.23± 2.98	-25.60 ± 3.55
-TAS	5.13 ± 0.05	5.13 ± 0.05
ΔG_{bind}	-30.10±2.98	-20.48 ± 3.55
	1B38 – Musbat Gossipol	
ΔH	-45.87± 2.83	-32.46 ± 4.67
-TAS	6.08 ± 0.04	6.08 ± 0.04
ΔG_{bind}	-39.79 ± 2.83	-26.38 ± 4.67
	1B38 – Manfiy Gossipol	
ΔH	-42.28± 4.47	-33.73 ± 5.45
-TAS	6.49 ± 0.04	6.49 ± 0.04
ΔG_{bind}	-35.79 ± 4.47	-27.23 ± 5.45

Xulosa va takliflar. Umuman olganda, olingan natijalar gossipoldagi ikkita izomer shakllarning nishon oqsillariga qarshi teng ta'sirga ega degan xulosaga olib keladi. Ikkala izomer ham oqsilning faol markazida o'xshash pozitsiyalarni egallashi mumkin

bo'lsa-da, ular bir xil aminokislota qoldiqlari bilan o'zaro ta'sir qilmaydi, bu esa faollikdagi kuzatilgan farqlarni tushuntirishi mumkin. Ularning turli xil farmakologik hatti-harakatlarining molekulyar asoslarini aniqlashtirish uchun turli xil MD simulyatsiya texnikalari va oqsil-ligandni baholash usullaridan foydalangan holda MGp va PGp ning keng qamrovli qiyosiy tadqiqotlari zarur bo'ladi.

Ushbu tadqiqot O zR FA BOKI Kompleks birikmalar laboratoriyasi byudjetdan moliyalashtirish tadqiqotlari doirasida bajarildi.

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