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TECHNOLOGY OF GENETIC ENGINEERING OF INSULIN FROM MICROORGANISMS

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KEYWORDS
recombinant insulin, E. coli, Saccharomyces cerevisiae, transgenic organism

ABSTRACT
The rapid growth in the number of diabetic patients and alternative insulin delivery methods such as inhalation or oral administration based on high doses are likely to increase the demand for recombinant insulin in the near future. Existing production technologies cannot meet the demand for affordable insulin due to limited production capacity and high production costs. This requires studying the mechanisms of production of therapeutic recombinant proteins. Recombinant human insulin is mainly used for therapeutic use of insulin produced by E. coli and Saccharomyces cerevisiae.

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MIKROORGANIZMLARDAN GEN MUHANDISLIGI YORDAMIDA INSULIN OLISH TEXNOLOGIYASI

KALIT SO‘ZLAR/
КЛЮЧЕВЫЕ СЛОВА:

Rekombinant insulin, E. coli, Saccharomyces cerevisiae, Transgen organizm

ANNOTATSIYA/ АННОТАЦИЯ

Diabetga chalingan bemorlar sonining tez o‘sishi va insulinni yuborishning muqobil usullarini, masalan yuqori dozalarga tayanadigan nafas olish yoki og‘iz orqali yuborish, yaqin kelajakda rekombinant insulinga bo‘lgan talabni oshirishi mumkin. Hozirgi ishlab chiqarish texnologiyalari ishlab chiqarish quvvatlarining cheklanganligi va ishlab chiqarish narxining yuqoriligi sababli arzon insulinga bo‘lgan talabni qondira olmaydi. Terapevtik rekombinant oqsillarni ishlab chiqarish mexanizmlarini o`rganish talab qiladi. Rekombinant inson insulinni asosan E. coli va Saccharomyces cerevisiae yordamida ishlab chiqarilgan insulinni terapevtik foydalanish uchun qo‘llaniladi.

Mavzuning dolzarbligi: oshqozon osti bezi Langergans orolchalarining β-hujayralari tomonidan ishlab chiqariladigan gormondir. Oshqozon osti bezi aralash sekretsiya bezi bo‘lib, oshqozon va taloq orqasida qorinning yuqori qismida ko‘ndalang joylashgan. Insulin oshqozon osti bezi tomonidan ishlab chiqariladigan peptid gormon bo‘lib, organizmdagi uglevodlar va yog‘lar almashinuvining markaziy regulyatori hisoblanadi [1, 28].

Qandli diabet bilan kasallanish juda katta sur’atda o‘sib bormoqda va 2025- yilga kelib butun dunyo bo‘ylab diabetga chalinganlar soni taxminan 300 millionga ko‘tarilishi taxmin qilinmoqda [2 29]. Inson insulini 51 aminokislotadan iborat va molekulyar og‘irligi 5808 daltonni tashkil qiladi. Insulin oshqozon osti bezi β-hujayralarida preproinsulin deb nomlanuvchi yagona polipeptid sifatida sintezlanadi. Preproinsulin 24-qoldiqli signal peptidini o‘z ichiga oladi, u yangi paydo bo‘lgan polipeptidni endoplazmatik retikulumga yo‘naltiradi. Signal peptidi polipeptid endoplazmatik retikulumning odamga o‘tishi natijasida proinsulin hosil bo‘liadi. Endoplazmatik retikulumda proinsulin 3 ta disulfid bog‘lanish hosil bo‘lishi bilan to‘g‘ri hosil bo`lishi bilan sodir bo`ladi [1, 3, 31].

Insulinning alohida olingan zanjirlari biologik faollikka ega emas. Insulin metabolizmning asosiy regulyatori hisoblanib, turli to`qimalarda glikogen sintezi va glyukoza sarf bo`lishini boshqaradi, oqsillar va yog`lar sintezini stimullaydi. Insulinning qondagi transport shakli erkin va asosan α-globulinlar bilan bog`langan. Insulin sintezi teskari aloqa yo`li bilan glyukoza tomonidan boshqariladi, uning yetishmasligi qandli diabetni keltirib chiqaradi [4, 30, 32 ]. Kasallikni asosiy belgisi – qonda glyukoza miqdorini ko`payib ketishi, ya`ni – giperglikemiya, glikogen sintezi susayadi, lipoliz va glyukoneogenez tezlashadi. Ta`sir mexanizmi quyidagi zanjir bo`yicha amalga oshiriladi: Geksokinaza → insulin → membrana retseptorlari – sial kislotasi - Na^+, K^+ATF-sintetaza, sAMF aktivatsiyasi

[5, 33].

Ishning maqsadi: E. coli va Saccharomyces cerevisiae mikroorganizmlari asosida inson inslulini ishlab chiqarish texnologiyasini o`rganish.

Material va metodlar: E. coli va Saccharomyces cerevisiae rekombinant oqsillarni keng miqyosda ishlab chiqarish uchun afzal mikroorganizmdir. Biroq, bir qator kamchiliklar uni recombinant oqsil biofarmatsevtikada ishlab chiqarish uchun kerakli texnologiyadir [6]. Biologik faollik uchun juda muhim bo‘lgan glikozillanish, fosforillanish, proteolitik ishlov berish va disulfid aloqalarining shakllanishi kabi turli post-translatsion modifikatsiyalar E. coli da sodir bo‘lmaydi [7, 34].

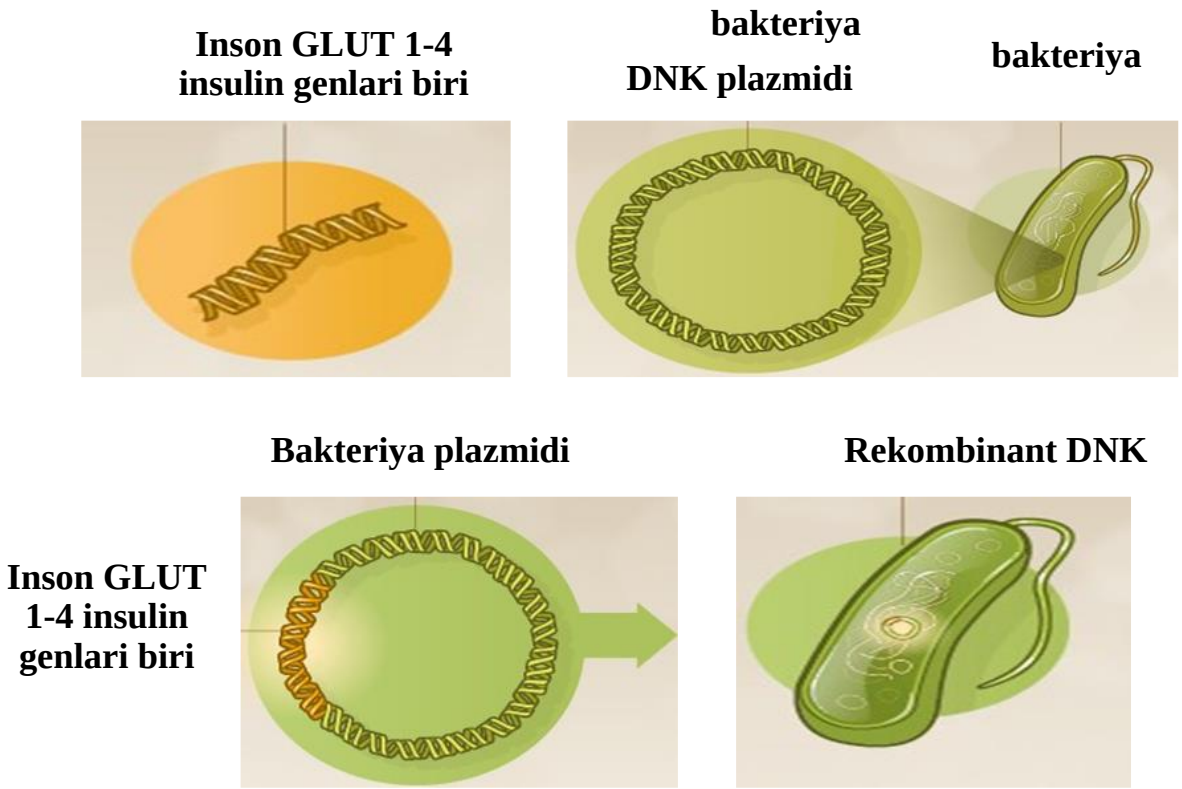
Olingan natijalar va ularning taxlili: Rekombinant DNK- bu oddiy bakteriyaning genetik materialiga inson genini kiritish imkonini beruvchi olimlar tomonidan ishlab chiqarilgan texnologiya hisoblanadi [8]. Ushbu rekombinant mikroorganizm inson geni tomonidan kodlangan oqsilni ishlab chiqaradi [9]. Prokariotlar orasida Escherichia coli har doim rekombinant oqsillarni ishlab chiqarish uchun afzal hisoblanadi, chunki u tezlik bilan ko`payishi, oddiy muhit talab qilishi, ishlov berish osonligi, yuqori mahsuldorlik va juda tejamkor kabi bir qator afzalliklarga ega bo`lib, rekombinat DNK asosida bakteriyalar bilan kompleks hosil qiladi [10, 35].

Rekombinant DNK asosida insulin olish texnologiyasi bosqichlari quyidagilardan iborat. Dastlab E. coli dan mos vektor (plazmid) ajratib olinadi, so‘ngra u endonukleaza fermenti yordamida kesiladi. Ajratib olingan gen (ya‘ni, insulin kodlovchi gen) β-hujayradan ajratiladi va ochilgan plazmidga kiritiladi [11, 38].

Plazmid va insulin genlari DNK ligaza fermenti tomonidan birgalikda rekombinatsiya qilingandan so`ng, ushbu rekombinatsiyalangan plazmid tegishli xost hujayraga (ya`ni *E. coli* yoki *Saccharomyces cerevisiae*) kiritiladi va endi bu rekombinatsiyalangan xost hujayrasi insulin gormonini sekretsiyasini oshirilishini ta`minlaydi. Hakura va shogirdlari (1977) ikkita zanjirlari insulinning DNK ketma-ketligini kimyoviy sintez qiladi va ikkita PBR322 plazmid vektoriga alohida kiritadi (boshqa plazmidlarni ham olish mumkin) [12, 39]. Bu gen plazmidning β-galaktosidaza genining yon tomoniga kiritiladi. Keyin rekombinant plazmid alohida E. coli bakteriyasida faoliyat yurita boshlaydi. Rekombinant proinsulin zanjirlarini hosil bo`ladi hamda ishlashga tayyor holatga keladi. Birlashtirilgan β-galaktosidaza-A zanjiri va β-galaktosidaza-B-zanjirlari alohida hosil bo`ladi. Ushbu proinsulin zanjirlari sianogen bromid bilan ishlov berish orqali β-galaktosidazadan ajratilgan holga keltirilib, proinsulin zanjirlarini β-galaktosidazadan ajratish mumkin, chunki zanjirlari uchun har bir genning N-terminalida qo‘shimcha kodon shaklidagi metionin aminokislotasi qo‘shilishidan hosil bo`ladi. Ajratilgandan so‘ng zanjirlari peptid zanjirlarini natriy disulfonat va natriy sulfit bilan sulfonlash reaksiyasi orqali oddiy insulinni tiklash uchun *in vitro* sharoitida olib boriladi [13, 40]. Rekombinant DNK texnologiyasi bilan insulin ishlab chiqarishning yana bir usuli Gilbert va Villokomaroff tomonidan ishlab chiqilmoda [15]. Bu usulda pre-proinsulin uchun mRNK Langergans oralchalari hujayralaridan ajratiladi. mRNK DNK hosil qilish uchun teskari transkripsiya

qilinadi va keyin penitsillinaza genining oʻrtasida joylashgan PBR322 plazmidiga kiritilib, keyin rekombinant plazmid ya`ni *E. coli* hujayrasini ishlashini ta`minlaydi. Rekombinant DNK penitsillinaza fermenti pre-proinsulin ishlab chiqarishni boshlovchi markerlardir [14].

Insulin ishlab chiqarish texnologiyasi biotexnologiya mahsulotlarini ishlab chiqarishning barcha asosiy bosqichlarini o'z ichiga oladi. Natijada, kristalining yakuniy mahsuloti bo'lib, u keyinchalik I va II diabet kasalliklarining og'ir shakllarini davolashda ishlatiladigan in'ektsion eritmalar tayyorlash uchun ishlatiladi. insulin ishlab chiqarish texnologiyasi biotexnologik mahsulotlar ishlab chiqarish barcha asosiy bosqichi o'z ichiga oladi. Natijada, kristalining yakuniy mahsuloti bo'lib, u keyinchalik I va II diabet kasalliklarining og'ir shakllarini davolashda ishlatiladigan in'ektsion eritmalar tayyorlash uchun ishlatiladi. Tanadagi bu gormonning asosiy ta'siri qon tarkibidagi glyukoza miqdorini pasayishi bilan namoyon bo'ladi [15, 16, 17]. Insulin ishlab chiqarish bosqichlari quyidagicha bo'ladi: Suv va havoni tayyorlash, tozalash, sanoat binolarini tozalash va jihozlarni sterilizatsiya qilish, xodimlarni tekshirish, qo'llarni qayta ishlash va steril poyafzal va kiyimlarni ishlab chiqarish kabi ishlar amalga oshiriladi.



1 rasm. Insulin genlari asosida toza holda insulin garmoni sintez qilish yo`li (texnologik sxema)

Bundan tashqari, dastlabki bosqichda, insulin oqsilni hosil qiladigan molekulalarning zanjirlarining asosiy kimyoviy sintezi amalga oshiriladi. A zanjiri 21 ta aminokislota

qoldiqlarini o'z ichiga oladi va B zanjiri 30 aminokislotadan iborat [18, 36].

ozuqa eritmalarini va hujayra kulturasini tayyorlash. Jonli hujayradan kerakli birikma hosil qilish uchun tegishli gen kiritiladi. Buning uchun plazmid maxsus fermentlar -restiriktazalar bilan kesiladi va kerakli birikmalarning sintezini kodlovchi genlar kiritiladi. Keyin, mikroineksiya usuli yordamida, o'zgartirilgan plazmid hujayra qaytariladi.

hujayra suspenziyasini yetishtirish: genetik yo`l bilan modifikatsiyalangan hujayralar ozuqa eritmasiga joylashtirilib, o'simliklar va reproduktiv o'simliklar uchun zarur bo'lgan barcha tarkibiy qismlarga ega va sterilizatsiya qilinadi, kulturani yetishtirish oldindan tozalangan maxsus bioreaktorlar bilan amalga oshiriladi. Reaktorga ma'lum miqdorda ozuqa eritmasi vaqti-vaqti bilan qo'shiladi va ayni paytda hujayra suspenziyasining bir xil miqdori olinadi [37].

kulturani taqsimlash (ajratish): Suyuqlik va hujayra kulturasini ajratish maxsus sedimentatorlarda (sedimentatsiya) usuli bilan, keyinchalik filtrlash orqali amalga oshiriladi, bu hujayralarning butunligini saqlab qolishga imkon beradi.

moddaning xromatografik tozalanishi: Turli usullar, xususan, frontal, anion almashinuvi va gel xromatografiyasidan foydalangan holda tegishli uskunalar bo'yicha amalga oshiriladi [27].

oqsil molekulasini olish: Aslida biotexnologik sintez bosqichi insulin tayyor molekulalari emas va uning zanjirlaridagi ikkita qismini tashkil etadi. Ular zanjirlarni oksidlanish va qatlamlardan so'ng tikiladi va ular disulfid ko'prigini shakllantiradi. Maxsus o'choqda sublimatsiya quritilishi, natijada olingan kristalli preparat standartga muvofiq, paketlangan, etiketli va iste'molchiga jo'natiladi [21, 22, 23, 24, 25, 26].

Xulosa: Hayvonlardan biosintetik, genetik muhandislik, modifikatsiyalangan gen muhandislik yo`llari asosida olingan insulin ishlab chiqarish yo`lga qo`yilgan lekin modifikatsiya asosida olingan insulinga talab juda kattadir. *E. coli* yoki *Saccharomyces cerevisiae asosida insulin olish yangi texnologiya hisoblanib, h*ozirgi kunda butun dunyoda genetik muhandislik yo`li bilan olingan insulin ulushi 2021 yilda bu ko'rsatkich 90%dan oshdi.

Foydalanilgan adabiyotlar ro`yxati

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