

Construction of mutant heparinase I with significantly increased specific activity.

Anna Kalinina¹, Larisa Borshchevskaya¹, Elena Patrusheva¹, Tatiana Gordeeva², and Sergey Sineoky¹

¹NRC "Kurchatov Institute"

²NRC "Kurchatov Institute" – GosNIIGenetika, Genomic Center

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Abstract

The cleavage of heparin by heparin lyases showed great potential as a cost-effective and innocuous method for producing heparin with low molecular weight (LMWH). One of the most studied and sought heparin lyase is heparinase I (HepI). However, the industrial use of HepI was largely hampered by its low specific activity and thermal stability. In this article we describe increasing in specific heparinase I activity by stepwise site-directed mutagenesis. Thus after two cycles of mutagenesis, we obtained mutant heparinase I *Flavobacterium heparinum* with significantly increased specific activity (25%).

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FH	MKKQILYLIVLQQLFCSAYAQKKSGNIPYRVNVQADSAKQKAIIDNKWVAVGINKPYA	60
BT	-----MUTAQTNTQTLMLPITERVNVQADSARINQIIDGCTVAVGTNKPHA	46
FH	LCYDDKLRNNGKPSYRFEKKAEDNSLEGYAAGETKGRTELSYSYATINDFKKFPSPVYQN	120
BT	LCRDFTNLDDGKPSYRFEKKTEDNLEGYAKGETKGRMEFSYCYATSDDERGLPADVYCK	106
FH	ACKLKTVYHYGKGCICOGSSRSYTFSVYIPSSFPDNATTFIAQWHGAPSRTLVATPEGET	180
BT	ACITKTVYHHGKCAFCOGSSRDYEFVSYPSSLDENVSTIFAQWHGMPDRTLVTQEGEV	166
FH	KTLSEIEFLALYDRMIEFKKNTAHDKVE-----KKDKDGKITVAGKPNGWKEQGGY	232
BT	KKLTVDDEFVELEKTTBFKKNVGHEKVARLDKQGNPVDKNGKPVVKAGKPNGWLEQGGY	226
FH	FLAFGFSKGYFYIKANSROWLTDKADRNANPENSEVMKEYSSEYKASTIAYKLPFAQ	292
BT	FLAFGFSKGLFYIKANSRRLWLDKDDRONANPCKTFVMKELTSEYKASTIAYKLPFAD	286
FH	FPKDCWITEDVAIDWTKYGKEANTILKPGKLDVMMTYTKNKKPQKAHIVNQOEILIGRND	352
BT	FPKDCWITFRVHIDWTVYGKEAETIVKPGMLDVRMDYQEQGKVKSKHIVDNEKILIGRND	346
FH	DDGYFFKFGIYRVGNSTVPVITYNLSGYSETAR	384
BT	DDGYFFKFGIYRVGDSVPVFCYNLAGYSER--	376

MKKQILYLIVLQQLFLCSAYAQQKKSGNIPYRVNVQADSAKQSEIIDNKWVAVGINKPY
 ALQYDDKLRFNGKPSYRFELKAEDNSLEGYAAGETKGRIELSYSYATTNDFKKFPSPVY
 QNAQKLKTVYHYGKGI**CEQGSSRSY**TFSVYIPSSFPDNATTIFAQWHGAPSR TLVATPEG
 EIKTLSIEEFLALYDRM**IFKKNIA****DKVEKKDKD**GKITYVAGKPNGWKVEQGGYPPLAF
 GFSKGYFYIKANSRQWLTDKADRNNANPENSEVMKPYSSSEYKTSTIAYKMPFAQFPK
 DCWITFDVAIDWTKYGKEANTILKPGKLDVMMTYT**KNKKPQK**AHIVNQEILIGRND**D**
DGYYFKFGIYRVGNSTVPVTYNLSGY**SETAR**



