MODIFIKASI PEPTIDA INHIBITOR α-AMILASE DARI TEH HITAM (*Camellia sinensis*), STUDI AKTIVITAS DAN KINETIKANYA

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INTISARI

Inhibisi terhadap α-amilase merupakan salah satu pendekatan terapeutik dalam pengelolaan diabetes melitus tipe 2, dengan akarbosa sebagai obat yang umum digunakan. Namun, penggunaannya sering menimbulkan efek samping gastrointestinal, sehingga diperlukan kandidat inhibitor baru yang lebih aman seperti peptida bioaktif. Penelitian ini bertujuan untuk memodifikasi peptida bioaktif hasil isolasi dari teh hitam (Camellia sinensis) guna meningkatkan aktivitas inhibisinya terhadap enzim α-amilase serta mempelajari karakter kinetik penghambatannya.

Penelitian diawali dengan pemilihan tiga peptida bioaktif dari teh hitam berdasarkan sifat fisikokimia menggunakan ExPASy ProtParam. Dari hasil prediksi interaksi pada PepSite2, peptida "CGKKFVR" dipilih karena memiliki afinitas paling baik terhadap α-amilase. Selanjutnya dilakukan penambahan molekul menggunakan HADDOCK dan analisis energi ikatan dengan PRODIGY. Modifikasi struktur dilakukan melalui *alanine screening* untuk menentukan residu tidak esensial, kemudian Cys-1 dan Gly-2 diganti menjadi Lys-1 dan His-2 sehingga diperoleh peptida modifikasi "KHKKFVR". Peptida asal dan hasil modifikasi disintesis dengan metode *solid-phase peptide synthesis* (SPPS). Uji aktivitas *in vitro* dilakukan menggunakan sistem S (amilum, peptida, α-amilase), S_0 (amilum, peptida), blanko (amilum, α-amilase), dan kontrol (amilum). Persentase inhibisi diukur dengan spektrofotometer UV-Vis 580 nm, nilai IC_{50} ditentukan melalui metode probit pada variasi konsentrasi 5–50 ppm, dan mekanisme inhibisi dianalisis dengan plot Lineweaver–Burk untuk memperoleh K_m dan V_{max}.

Hasil *in silico* menunjukkan bahwa peptida "KHKKFVR" memiliki afinitas lebih tinggi (HADDOCK score −128,1 ± 4,3; energi ikatan −9,0 kcal/mol) dibandingkan "CGKKFVR" (−97,8 ± 3,8; −6,8 kcal/mol). Secara *in vitro*, "KHKKFVR" memiliki nilai IC_{50} lebih rendah (12,37 ± 0,16 µM) dibandingkan "CGKKFVR" (16,14 ± 0,19 µM). Analisis kinetika menunjukkan peningkatan K_m dari 3597,6 menjadi 3639,6 µg/mL dan penurunan V_{max} dari 243,90 menjadi 192,31 µg/mL·menit^{-1}, menandakan mekanisme inhibisi campuran. Hasil ini menegaskan bahwa modifikasi residu peptida mampu meningkatkan efektivitas inhibisi α-amilase serta memperkuat pemahaman mengenai mekanismenya.

Kata kunci: α-amilase, diabetes melitus, *in silico*, *in vitro*, peptida bioaktif

MODIFICATION OF α-AMYLASE PEPTIDE INHIBITORS FROM BLACK TEA (Camellia sinensis), STUDY OF THE ACTIVITY AND ITS KINETICS

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ABSTRACT

Inhibition of α-amylase is one of the therapeutic approaches in managing type 2 diabetes mellitus, with acarbose being the commonly used drug. However, its use often causes gastrointestinal side effects, necessitating the search for safer alternative inhibitors such as bioactive peptides. This study aims to modify bioactive peptides isolated from black tea (*Camellia sinensis*) to enhance their inhibitory activity against α-amylase and to investigate their kinetic inhibition characteristics.

The research began with the selection of three bioactive peptides from black tea based on their physicochemical properties using ExPASy ProtParam. From the interaction prediction on PepSite2, the peptide "CGKKFVR" was selected due to its strong affinity toward α-amylase. Molecular modification was then performed using HADDOCK, followed by binding energy analysis with PRODIGY. Structural modification was conducted through *alanine screening* to identify non-essential residues, after which Cys-1 and Gly-2 were substituted with Lys-1 and His-2, resulting in the modified peptide "KHKKFVR." Both the native and modified peptides were synthesized using the *solid-phase peptide synthesis* (SPPS) method. *In vitro* activity tests were conducted using the S system (starch, peptide, α-amylase), S_0 (starch, peptide), blank (starch, α-amylase), and control (starch). The inhibition percentage was measured using a UV-Vis spectrophotometer at 580 nm, while the IC_{50} values were determined by the probit method across concentration variations of 5–50 ppm. The inhibition mechanism was analyzed using Lineweaver–Burk plots to determine K_m and V_{max} values.

In silico analysis showed that the peptide "KHKKFVR" exhibited stronger binding affinity (HADDOCK score −128,1 ± 4,3; binding energy −9,0 kcal/mol) compared to "CGKKFVR" (−97,8 ± 3,8; −6,8 kcal/mol). *In vitro* results also indicated that "KHKKFVR" had a lower IC_{50} value (12,37 ± 0,16 µM) than "CGKKFVR" (16,14 ± 0,19 µM). Kinetic analysis revealed an increase in K_m from 3597,6 to 3639,6 µg/mL and a decrease in V_{max} from 243,90 to 192,31 µg/mL·min^{-1}, indicating a mixed-type inhibition mechanism. These findings confirm that peptide residue modification successfully enhances α-amylase inhibitory effectiveness and provides a deeper understanding of its inhibitory mechanism.

Keywords: α-amylase, bioactive peptides, diabetes mellitus, in silico, in vitro.