IDENTIFIKASI PEPTIDA INHIBITOR α-AMILASE DARI OVALBUMIN DENGAN PENDEKATAN *IN SILICO DIGESTION* DAN PENAMBATAN MOLEKUL, SERTA UJI AKTIVITASNYA

Talitha Anindya Nariswari
21/480331/PA/20861

INTISARI

Ovalbumin merupakan protein utama dalam putih telur dan dapat menjadi sumber peptida bioaktif melalui proses hidrolisis. Ovalbumin dapat dimanfaatkan sebagai sumber peptida antidiabetes inhibitor α-amilase. Proses identifikasi peptida bioaktif berupa penentuan urutan asam amino melibatkan serangkaian pemurnian hidrolisat protein yang panjang. Alternatif identifikasi peptida bioaktif dari hidrolisat protein adalah dengan pendekatan *in silico* yang mencakup *in silico digestion* dan penambatan molekul. Penelitian ini bertujuan menentukan urutan asam amino peptida dari hidrolisat ovalbumin *in silico* yang mempunyai aktivitas inhibisi terhadap α-amilase.

Hidrolisis secara *in silico digestion* dilakukan terhadap urutan asam amino ovalbumin beberapa spesies hewan kelas burung (*aves*) dengan peladen Expassy PeptideMass untuk menemukan urutan asam amino peptida. *Scoring* dilakukan dengan PeptideRanker dan PepSite2 untuk melihat bioaktivitas peptida. Penambatan molekul dengan HADDOCK menyajikan prediksi interaksi antara molekul peptida dengan α-amilase (kode PDB: 1PIF). Peptida bioaktif hasil penambatan molekul terpilih akan dilakukan sintesis dan uji aktivitas. Uji aktivitas dilakukan untuk melihat adanya potensi peptida dalam menginhibisi α-amilase. Kinetika inhibisi peptida ditentukan melalui Lineweaver-Burk *plot*.

Hasil hidrolisis dengan *in silico digestion* menghasilkan 124 peptida. Hasil *screening* awal diperoleh peptida dengan urutan “ADHPFLFCVK” dan “VDHPFLYCIK” diprediksi memiliki sifat sebagai inhibitor α-amilase berdasarkan hasil *scoring* dengan nilai >0,5 pada PeptideRanker dan nilai p situs pengikatan potensial peptida <0,01 pada PepSite2. Hasil penambatan molekul peptida “ADHPFLFCVK” dan “VDHPFLYCIK” diperoleh sebanyak 4 dan 2 interaksi hidrogen konvensional yang diantaranya terdapat interaksi dengan situs katalitik α-amilase, serta nilai afinitas energi ikat (ΔG) sebesar -9,5 dan -9,6 kkal mol^{-1}. Hasil uji aktivitas menunjukkan peptida menginhibisi α-amilase secara moderat dengan nilai IC_{50} untuk peptida "ADHPFLFCVK" dan "VDHPFLYCIK" sebesar 38,84±0,48 dan 51,16±4,98 µM. Studi kinetika terhadap peptida “ADHPFLFCVK” menunjukkan bahwa tipe inhibisi campuran dengan nilai K_m sebesar 1907,5±372,4 µg mL^{-1} dan V_{maks} sebesar 284,5±40,5 µg mL^{-1} min^{-1}.

Kata kunci: amilase*, in silico digestion*, ovalbumin, penambatan molekul, peptida

IDENTIFICATION OF α-AMYLASE INHIBITORY PEPTIDES FROM OVALBUMIN USING IN SILICO DIGESTION AND MOLECULAR DOCKING, AND ITS ACTIVITY STUDY

Talitha Anindya Nariswari
21/480331/PA/20861

ABSTRACT

Ovalbumin, the primary protein in egg whites can be a source of bioactive peptides through hydrolysis. An α-amylase inhibitor antidiabetic peptides can be utilized from ovalbumin. Traditionally, identifying bioactive peptides involves a laborious purification process of protein hydrolysates to determine their amino acid sequences. An alternative method for the identification of bioactive peptides from protein hydrolysates is through in silico approaches that include in silico digestion and molecular docking. This research aims to use in silico methods to identify and characterize ovalbumin-derived peptides with α-amylase inhibitory activity, focusing on determining their amino acid sequences.

In silico digestion hydrolysis was conducted on ovalbumin primary structure of animal species from the class Aves using the Expassy PeptideMass server. The resulted peptided was then screended by scoring using PeptideRanker and PepSite2 to assess the bioactivity of the peptides. Predictions of interactions between the peptide molecules and α-amylase (PDB ID: 1PIF) were provided by molecular docking using HADDOCK. Based on the docking results, selected bioactive peptides will undergo synthesis and activity testing to evaluate their potential as α-amylase inhibitors. The inhibitory kinetics of these peptides will be further characterized using Lineweaver-Burk plots to determine their mechanism of action.

The in silico digestion ovalbumin produced by 124 peptides. Peptides with sequences 'ADHPFLFCVK' and 'VDHPFLYCIK' were predicted to have properties as α-amylase inhibitors based on scoring results with values >0.5 on PeptideRanker and potential peptide binding site p-values <0.01 on PepSite2. The molecular docking revealed that the peptides "ADHPFLFCVK" and "VDHPFLYCIK" have 4 and 2 conventional hydrogen bond interactions to the α-amylase (PDB ID: 1PIF) respectively, including interactions to the catalytic site. The binding energy affinity values (ΔG) of of the interations was -9.5 and -9.6 kcal mol^{-1} respectively. The α-amylase inhibitor activity of the peptides show moderate activity with IC_{50} values for peptides "ADHPFLFCVK" and "VDHPFLYCIK" of 38.84±0.48 and 51.16±4.98 µM respectively. It was shown by kinetic studies on peptide "ADHPFLFCVK" that the type of inhibition is mixed inhibition with K_m value of 1907.5±372.4 µg mL^{-1} and V_{maks} of 284.5±40.5 µg mL^{-1} min^{-1}.

Keywords: amylase, in silico digestion, molecular docking, ovalbumin, peptide