

Isolasi dan karakterisasi neuroglobin dan enzim pereduksi metneuroglobin (neuroglobin Fe^{3+}) dari jaringan otak sapi = Isolation and characterization of neuroglobin and the reducing enzyme metneuroglobin (neuroglobin Fe^{3+}) from bovine brain tissue

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Abstrak

Oksigen merupakan zat penting dalam metabolisme energi tubuh, terutama otak. Neuroglobin (Ngb), salah satu famili hemoprotein berperan sebagai pengikat oksigen di otak. Seperti hemoprotein lainnya, gugus hem pada Ngb rentan mengalami oksidasi Fe^{2+} menjadi Fe^{3+} yang dapat menghilangkan fungsi pengikatan oksigennya. Penelitian ini bertujuan untuk menganalisis perubahan spektrum Ngb terhadap oksigen dan karbonmonoksida serta mencari potensi enzim yang dapat mereduksi Ngb teroksidasi (metNgb). Protein Ngb diisolasi dengan teknik fraksinasi menggunakan amonium sulfat kejenuhan 90%, dipurifikasi dengan kromatografi penukar anion (DEAE Selulosa) dan kromatografi imunoafinitas. Hasil isolasi dikonfirmasi dengan SDS-PAGE dan Western blot. Enzim pereduksi metneuroglobin diisolasi dengan RIPA lysis buffer, dipurifikasi dengan kromatografi Affi gel blue, dan dikonfirmasi dengan SDS-PAGE. Ngb hasil purifikasi memiliki berat molekul 17,26 kDa. Analisis spektrum pada rentang panjang gelombang 350-500nm, memperlihatkan puncak sorot dari deoksiNgb, oksaNgb, karboksiNgb dan metNgb berturut-turut adalah 415 nm, 405 nm, 405 nm, dan 420 nm. Hasil isolasi enzim pereduksi yang diperoleh terdiri dari 2 bagian, yaitu eluat tidak terikat matriks (eluat-1) dan eluat terikat matriks (eluat-2). Hasil SDS-PAGE dari eluat-1, eluat-2 dan fraksi bebas Ngb (hasil samping purifikasi Ngb) menunjukkan 3 pita yang sama pada berat molekul 72,45; 26,84 dan 16,33 kDa yang diduga berpotensi sebagai enzim pereduksi. Kinetik reduksi diuji dengan mereaksikan fraksi dan metNgb serta mengukur serapan deoksiNgb yang terbentuk tiap satuan waktu. Hasil pengukuran rasio $NgbFe^{3+}$ menjadi $NgbFe^{2+}$ dari fraksi bebas Ngb, eluat-1 dan eluat-2, yang memiliki aktivitas reduksi terbaik adalah eluat-1 karena memiliki nilai regresi terbaik.

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reduction kinetics was tested by reacting the fraction and metNgb and measuring the deoxyNgb uptake formed per unit time. The results of the measurement of the ratio of NgbFe³⁺ to NgbFe²⁺ from the free fractions Ngb, eluate-1 and eluate-2, which has the best reduction activity is eluate-1 because it has the best regression value. Oxygen is an important substance in the body's energy metabolism, especially the brain. Neuroglobin (Ngb), one of the hemoprotein families, acts as an oxygen binder in the brain. Like other hemoproteins, the haem group in Ngb is susceptible to Fe²⁺ oxidation to Fe³⁺ which can eliminate its oxygen binding function. This study aims to analyze the changes in the Ngb spectrum towards oxygen and carbon monoxide and to find the potential for enzymes that can reduce oxidized Ngb (metNgb). Ngb protein was isolated by fractionation technique using ammonium sulfate 90% saturation, purified by anion exchange chromatography (DEAE Cellulose) and immunoaffinity chromatography. The isolation results were confirmed by SDS-PAGE and Western blot. The metneuroglobin-reducing enzyme was isolated by RIPA lysis buffer, purified by Affi gel blue chromatography, and confirmed by SDS-PAGE. The purified Ngb has a molecular weight of 17.26 kDa. Spectrum analysis in the wavelength range of 350-500nm, showed the afternoon peaks of deoxyNgb, oxyNgb, carboxyNgb and metNgb were 415 nm, 405 nm, 405 nm, and 420 nm, respectively. The results of the isolation of reducing enzymes obtained consisted of 2 parts, namely the matrix-bound eluate (eluate-1) and the matrix-bound eluate (eluate-2). SDS-PAGE results of eluate-1, eluate-2 and Ngb-free fraction (byproduct of Ngb purification) showed the same 3 bands at a molecular weight of 72.45; 26.84 and 16.33 kDa which are thought to have potential as reducing enzymes. The reduction kinetics was tested by reacting the fraction and metNgb and measuring the deoxyNgb uptake formed per unit time. The results of the measurement of the ratio of NgbFe³⁺ to NgbFe²⁺ from the free fractions Ngb, eluate-1 and eluate-2, which has the best reduction activity is eluate-1 because it has the best regression value