Pengaruh Amobilisasi-Kovalen Bromelain batang pada CNBr-Sepharose 4B terhadap aktivitas dan stabilitas enzim = the Effect of the stem Bromelan Immobilization by Covalent attachment onto CNBr-Sepharose 4B towards enzymatic activities and stabilities

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Abstrak

ABSTRAK
Ruang Lingkup dan Cara Penelitian : Pesatnya kemajuan bioteknologi menyebabkan peningkatan kebutuhan akan enzim sebagai agen biologi. Khususnya enzim proteinase kebutuhannya akan semakin meningkat karena fungsinya yang sangat luas. Akan tetapi pemakaian enzim secara konvensional dalam industri, selain tidak efisien dan efektif, juga berarti tidak menggunakan kemampuannya dengan optimum. Salah satu cara untuk memecahkan masalah stabilitas dan kebutuhan enzim tersebut ialah dengan menggunakannya dalam bentuk amobil. Dengan teknik ini, enzim dapat digunakan berulang kali atau terus menerus, sehingga peranannya dalam bidang biomedik; analisis; industri dan riset akan lebih luas. Pada penelitian ini telah dilakukan proses amobilisasi kovalen, menurut metoda modifikasi Porath dick, dengan bahan utama bromelain batang, bahan matriks CNBr-Sepharose 4B, kasein dan BApNA. Prosedur amobilisasi dilakukan dengan reaksi rangkai selama 16 jam pada suhu 4°C antara 10 mg protein enzim dengan 1 g bahan matriks, dalam larutan NaHC03 0,1 M pH 8,0, yang mengandung merkaptoetanol, EDTA dan NaCl. Pengamatan dilakukan terhadap : jumlah, kadar protein, aktivitas dan stabilitas enzim amobil.

Hasil dan Kesimpulan : Enzim amobil diperoleh dalam bentuk bubuk kering sebanyak 426,5 mg (rendemen 42,2%), yang mengandung 5,1 mg protein enzim. Aktivitas relatif terhadap kasein dan BApNA, didapatkan masing-masing sebesar 34,7% dan 62,4%. Pada uji stabilitas terhadap pemakaian berulang (N=10), diperoleh aktivitas-sisa 79,3% dan kadar proteinnya menurun sekitar 10%. Terhadap pengaruh pemanasan dan penyimpanan, enzim amobil memperlihatkan stabilitas yang lebih tinggi dibanding enzim bebas. Pra-inkubasi pada suhu 60°C selama 1 jam menyebabkan aktivitas-sisa 88,5%, sedangkan enzim bebas aktivitasnya tinggal 63,8%. Penyimpanan dalam bentuk suspensi selama 6 minggu pada suhu 4°C menyebabkan aktivitas-sisa 80,8%, sedang pada suhu kamar aktivitasnya menjadi 46,1%. Sementara kondisi yang sama menyebabkan aktivitas-sisa larutan enzim bebas, masing-masing menjadi 44,8% dan 9,1%.
<hr><i>ABSTRACT
The Effect Of The Stem Bromelain Immobilization By Covalent Attachment Onto CNBr-Sepharose 4B Towards Enzymatic Activities And StabilitiesScope and Method of Study: The rapid progress in biotechnology brings about the increase in needs for enzymes as a biological agent. The need for proteinase in particular, will increase much more because of its broad range in functions. However, the conventional use of enzyme in the industry, besides inefficient and ineffective, but also is not utilizing its potential as biocatalyst optimally. One way to solve the problem of the stability and the need for enzyme is by using them in immobilized form, through an immobilization process. With this technique, the enzymes can be use repeatedly or continuously, and hence its role in the field of biomedical, analysis, industry and research can be more extended. In this study a covalent-immobilization process has been carried out, according to the

modification method of Porath et al, by using stem bromelain, CNBr- Sepharose 413, casein and BApNA as main materials. The immobilization procedure was carried out through a coupling reaction for 16 hours at 4°C, between 10 mg enzyme and I g matrix in a solvent of 0.1 M NaHC03 , pH 8.0 that contains mercaptoethanol, EDTA and NaCl. The immobilization process was evaluated with respect to: the yield, protein concentration, activity and stability of the immobilized enzymes.

Finding and Conclusions: The immobilized enzymes were obtained in a dry powder form of 426.5 mg (yield of 42.2%) that contains 5.1 mg of protein. The relative activity towards casein and BApNA were obtained at 34.7% and at 62.4%, respectively. At reuse stability test (N=10), the activity is seen retained at 79.3 % and its protein content diminished about 10%. Under the influence of heat and storage, the immobilized enzymes, showed a higher stability compared to free enzymes: A pre-incubation at 60°C for 1 hour caused a residual activity of 88.5%, whereas for the free enzymes activity remains at 63.8%. The storage in a suspension form during 6 weeks at 4°C caused a residual activity of 80.8%, whereas at a room temperature the activity became 46.1 %. The corresponding activities of the free enzymes were 44.8% and 9.1%, respectively.</i>