Pemurnian enzim fosfatase alkali dari escherichia coli = The purification of alkaline phosphatase from e. coli

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Abstrak

Ruang Lingkup dan Cara Penelitian: Enzim fosfatase alkali antara lain digunakan dalam teknik ?enzyme immunoassay?, untuk mengukur kadar sesuatu zat dalam cairan tubuh dalam jumlah yang sangat kecil. Dalam penelitian ini diusahakan isolasi dan pemurnian enzim fosfatase alkali dari E. coli. Identifikasi kuman dilakukan dengan agar Endo, agar darah, tes pewarnaan Gram, sifat-sifat biokimia, dan tes serologik. Untuk pemurnian enzim digunakan sonikator, kromatografi pertukaran ion dengan DEAE Biogel, dan kromatografi gel dengan Sephadex G-100. Kemurnian enzim diperiksa dengan elektroforesis pada membran selulosa asetat. Aktivitas enzim secara kuantitatif ditentukan dengan spektrofotometer, dan secara kuaLitatif dapat dilihat dengan agar substrat. Kadar protein diukur dengan metoda Lowry. Terhadap fraksi gel diteliti pengaruh suhu, pH, ion logam, dan jenis bufer atas aktivitas enzim. Demikian pula ditentukan nilai Km dan Vmax, serta reaksi hidrolisis tanpa dan dengan transfosforilasi.

Hasil dan Kesimpulan: Kuman diidentifikasi sebagai E. coli non-patogen. Enzim diperoleh setelah fraksi gel dengan ,pemurnian 242 kali dan hasil 59%. Pada eLektroforesis ditemukan kadar protein enzim 52,8%. Enzim memiliki pH optimum 8,0, dan tidak stabil bila diinkubasi selama 1 jam diluar pH optimum. Aktivitas enzimeningkat secara Linier sampai suhu 45° C, dengan koefisien suhu 1,49. Enzim stabil pada inkubasi selama 20 menit pada suhu 25 - 45° C. Aktivitas enzim tidak dipengaruhi penambahan ion Mg dan Zn (0,01 M). Aktivitas meningkat dengan meningkatnya molaritas bufer, Vmax terbesar didapatkan dalam buffer Tris dan Km terkeciL pada bufer AMP. Reaksi hidrolisis dan transfosforilasi berlangsung pada bufer Tris dan AMP, sedangkan pada bufer glisin hanya terjadi reaksi hidrolisis.
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Scope and Method of Study: The enzyme alkaline phosphatase is used among others in enzyme immunoassay, to enable the quantitation of a small amount of substances in body fluids. In this study, an attempt was carried out for the isolation and purification of the enzyme from E. coli. The bacteria was identified through culture on Endo and blood agar, Gram staining, biochemical tests, and serology. The bacteria were disrupted by ultrasonication, and the enzyme purified by ion exchange chromatography on DEAE Biogel followed by gel chromatography on Sephadex G-100. Enzyme purity was examined by electrophoresis on cellulose acetate. Enzyme activity was determined by spectrophotometry, and protein concentration was measured by the method of Lowry. The gel fraction was tested for the effect of pH, temperature, metal ion, and type of buffer. The Km and Vmax was measured, for hydrolysis with and without transphosphorylation.

Findings and Conclusions: The bacteria was identified as non-pathogenic E. coli. After gel chromatography the enzyme was purified 242 fold, at 59%, yield. Electrophoresis revealed that the enzyme content was 52.8 %. The enzyme has a pH optimum of 8.0, and it was unstable on standing for 1 hour outside the pH

optimum. Enzyme activity increased Linearly with temperature (to 45° C), with a temperature coefficient of 1.49. The enzyme is stable for 20 minutes at 5° - 45++ C, and the activity not influenced by Mg++ and Zn++ ions (0.01 M). The activity increased with increased molarity of the buffer, the highest Vmax was observed with Tris buffer, and the Lowest Km with AMP buffer. Hydrolysis and transphosphorylation occurred in Tris and AMP buffer, while in glycine buffer only hydrolysis was observed.