Isolation and purification of *CeqI* endonuclease, an isoschizomer of *EcoRV*

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Corynebacterium equi produces a new restriction endonuclease, an isoschizomer of *EcoRV* (1). The bacterial strain does not contain significant levels of other nucleases, so the restriction endonuclease activity can be detected in crude extracts. The enzyme is purified by ammonium sulphate fractionation (50-70 % saturation) and DEAE cellulose chromatography (elutes with 200 mM KCl in PC buffer). The partially purified enzyme is perfectly suitable for cloning purposes. Further purification can be achieved on aminopentyl- or aminohexyl-Sepharose and on FPLC Mono Q columns.

Several thousand units of *CeqI* can be purified from one gram of bacterial cells. The enzyme is stable in elution buffer, and can be stored in the same buffer (containing 50 % glycerol) at -20°C. The enzyme needs relatively high salt concentration for maximal activity (optimum is between 100-200 mM) and the presence of salt protects the enzyme against denaturation at extreme pH (pH 5 and 10) and at high temperatures (up to 45°C). For cloning purposes the activity is measured in high salt buffer (6 mM trisHCl, pH 7.5, 6 mM MgCl₂, 6 mM beta-mercaptoethanol, 150 mM NaCl).

The enzyme was characterized by its cleavage patterns using pBR322 and lambda phage DNA. 5' terminal nucleotide of *CeqI* cut DNA fragments was determined by thin layer chromatography on PEI-cellulose after phosphatase treatment and polynucleotide kinase labelling and digestion by snake venom phosphodiesterase and DNase I (2), and proved to be adenine. Sequencing of the cleavage site was carried out by the Maxam and Gilbert method (3) and identified the cleavage sites as GAT ATC and CTA TAG on the two strands, respectively, like in the case of *EcoRV* (1).

Preliminary data show that the enzyme protein has rather unusual properties for a restriction endonuclease. Long ultracentrifugation sediments enzyme activity, a fact indicating aggregation or association of the enzyme to some kind of a structure. Experiments show that non-ionic detergents and organic solvents do not convert the enzyme into a lower molecular mass form, arguing against the possibility of membrane association of the enzyme.

Molecular mass of the native enzyme is over 300,000 d, measured by gel-chromatography in 100 mM KCl in PC buffer. Salt concentrations over 1 M reversibly dissociate the enzyme to a 40 kd, inactive form.

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