

NICKING ENDONUCLEASE IN CYANOBACTERIA

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Abstract

The presence of restriction endonuclease in cyanobacteria is well known but another class of enzyme, the nicking endonuclease, has not so far been reported. We have come across a putative nicking endonuclease from the unicellular cyanobacterium *Chroococcus minutus* and report this for the first time.

Keywords: Nicking endonuclease, Restriction endonuclease, Cyanobacteria, *Chroococcus minutus*

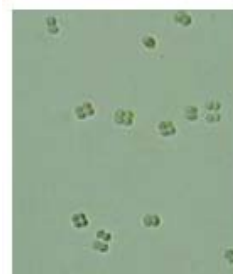
Introduction

Restriction endonucleases are enzymes that cleave the DNA strand at a specific site. A restriction endonuclease OfoI was reported from an Indian isolate *Oscillatoria foreau* for the first time by us (Saravanan *et al.*, 2003). Many more enzymes that cleave the DNA have also been discovered which include the Nicking endonucleases. The nicking endonucleases, unlike restriction endonucleases, cleave only one strand of the duplex DNA forming a nick or a gap. They are site specific and are involved in many DNA related metabolism. Restriction endonucleases have been identified in great numbers but very few nicking endonucleases have been isolated and only 13 enzymes are commercially available (Roberts and Macelis 2001). Abdurashitov *et al.* (1996) reported a natural nicking enzyme N.BstSEI from a bacterial source, *Bacillus sterothrophilus* SE589. Two other nicking enzymes N.BstNBI (Morgan *et al.*, 2000) and N.Bst9I (Dedkov *et al.*, 2001) were also identified from different strains of *B. sterothrophilus* and they were found to be isoschizomers of N.BstSEI. Xia *et al.* (1988) and Zang *et al.* (1998) indicated the presence of nicking endonuclease from a viral source. The lack of more number of nicking endonucleases perhaps indicates their limited natural occurrence. Many new nicking enzymes have been developed by engineering certain Type II restriction endonucleases. But there were no evidence for the presence of nicking endonuclease in cyanobacteria. The search for new nicking endonuclease is still in progress.

In the present study during the screening for restriction endonucleases in *Chroococcus minutus* (fig 1.), a unicellular cyanobacterium, we came across another enzyme which appeared to be unique and not known to be present so far in cyanobacteria. The enzyme was identified as a putative nicking endonuclease. In *C. minutus* the presence of neither a restriction endonuclease nor a nicking endonuclease is reported so

far. Here we report the presence of both. The isolate was obtained from the Culture Collection of Algae Centre for Advanced Studies in Botany, University of Madras, which holds the accession number *Chroococcus minutus*- A264. The cultures were grown in BG11 medium (Rippka *et al.*, 1979) and maintained under a light intensity of 30–40 $\mu\text{Em}^{-2}\text{S}^{-1}$ at $27^{\circ}\pm 1^{\circ}\text{C}$ with 12h alternate L/D condition. The growth was measured in terms of Chlorophyll *a* content (Mackinney, 1941).

Figure1. Colonies of *Chroococcus minutus* (x 400) grown in BG 11 medium



The enzyme was partially purified by two chromatographic steps using phosphocellulose and heparin sepharose column. 10g of cells were suspended in 20mL of buffer A (10 mM Tris pH 7.4, 50 mM NaCl, 0.1mM EDTA and 5 mM β Mercaptoethanol) and were subjected to sonication. The cells were disrupted and the homogenate was centrifuged at 95,000 g for 1h. The supernatant was passed through phosphocellulose column which was equilibrated with buffer B (10 mM potassium phosphate buffer pH 7.4, 50 mM NaCl, 0.1mM EDTA and 5 mM β mercaptoethanol). The sample was passed through the column and the column was then washed with buffer B and eluted using a linear salt gradient of 0.05–1.5M NaCl in buffer B. The active fractions were dialysed against buffer A and then loaded onto heparin sepharose column. The unbound proteins

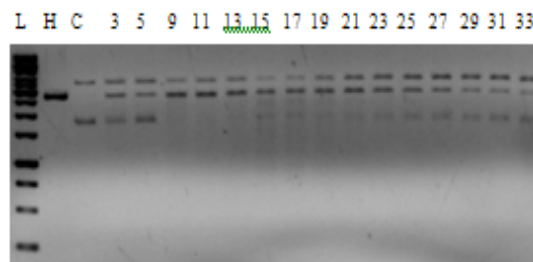
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were removed by washing the column using 500mL of buffer A and the bound proteins were eluted using 0.05–1.0M NaCl. The active fractions were pooled and dialysed against buffer A containing 50% glycerol and stored at -20°C. The endonuclease assays were done using a reaction mixture containing 0.5µg of pUC19 DNA as a substrate and NEB 4 reaction buffer. Reactions were done by incubating the enzyme with the reaction mixture for 1h at 37°C.

In the process of isolating a restriction endonuclease another enzyme was found to intervene, which was identified as a putative nicking endonuclease. The results clearly indicate the presence of both the endonuclease activity. The activity of the alternative fractions of phosphocellulose column was tested. Fractions 11–27 showed prominent linearization and faint nicking activity. In fractions 13 – 17 the enzyme was more active (fig 2.). Nicking activity was observed in fractions 21 – 33 and partial linearization was observed in all the fractions (fig 2.) (When the enzyme cuts a strand of a Super coiled DNA it is converted into an open circle form. So the appearance of an open Circle band and disappearance

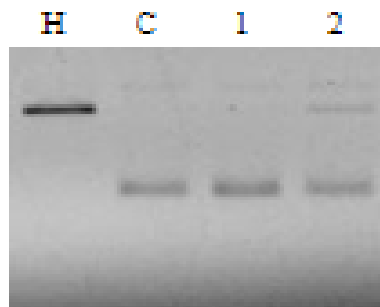
of the Super Coiled band indicates a clear nicking activity). The SDS – PAGE analysis also showed a difference in the two sets of active fractions. Similar results were obtained even after the second step of purification. The restriction digestion pattern again showed a mixture of both the activities. Though the purification process was efficient the contaminating nicking endonuclease (?) was still present which was confirmed by a SDS-PAGE profile. The endonucleases generally require metal ions for their activity, the presence and absence of Mg^{2+} in the reactions were studied. Different controls were made and the activity was compared. Three reactions were prepared i) without Mg^{2+} , ii) with EDTA, and iii) heat killed enzyme was used. The enzyme was not active in the absence of Mg^{2+} which clearly signifies that the enzyme requires Mg^{2+} for its activity (fig 3.). The other two reactions also did not show any endonuclease activity. Many authors have stated that when a restriction enzyme cleaves the circular DNA it is converted initially into an open circle form and later to the linear form. Hence by using a super coiled DNA, it is likely to determine the rate of reaction and the mode of action.

Figure 2. Endonuclease activity of the enzyme fractions from *Chroococcus minutus* after Phosphocellulose column



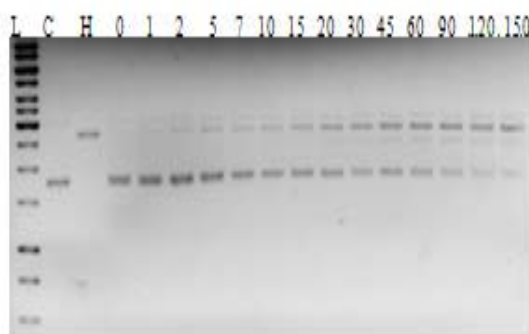
L- 1kb Ladder, H- Linear control pUC 19 digested with Hind III, C- Uncut pUC 19 DNA, Lanes 3–33: alternate enzyme fractions. Prominent linearization can be noted in fractions 11–27. The appearance of distinct open circle band and complete disappearance of the super coiled form are clearly observed in fractions 21–33 signifies the nicking activity (?) of the enzyme.

Figure 3. Assay on the endonuclease activity of the partially purified enzyme in the presence and absence of Mg^{2+}



H: Linear DNA obtained by digesting pUC 19 DNA with Hind III, C: Uncut pUC 19 DNA
Lane 1: Without Mg^{2+} Lane 2: With Mg^{2+}

Figure 4. A time course (minutes) on the endonuclease activity of the partially purified enzyme



L: 1Kb Ladder, C: Uncut pUC19 DNA, H: Linear control, pUC 19 DNA digested with Hind III. Lanes 0 – 150: Aliquots withdrawn at different time, as indicated, from the reaction mixture containing 7µg pUC19 DNA and the partially purified enzyme incubated at 37° C. A distinct linear band is observed after 15 min of incubation which is more prominent as the time period increased. A complete linearization can be observed after 120 min.

Certain type II restriction endonucleases behave like monomers which then dimerizes in the presence of cognate DNA and divalent metal ions. They cleave the two strands of DNA in a sequence manner. First strand being nicked and then further digested to form a linear band. Similar cleavage pattern was observed in a type IIS restriction enzyme P1eI. The intensity of the open circle band gradually reduced as the time increased forming linear band and only a faint nicked intermediate remained after 120 minutes (Higgins *et al.*, 2001). So, to check whether the newly isolated enzyme also behaves in such a manner, a time course on the activity of the enzyme was done. Only supercoiled pUC19 DNA was used as a substrate to clearly determine the nicking activity. The enzyme started linearising the DNA after 2 minutes which was prominent after 45 minutes. But the nicking activity did not decrease gradually and was observed in all the reactions (fig 4.). This suggests that the new enzyme isolated from *Chroococcus minutus* perhaps, possess both nicking and typeII restriction endonuclease activity. However, we have reported the observations made which signifies the presence of nicking endonuclease (?) but convincing evidence of its presence has to be further substantiated. The search for a new type II Restriction endonuclease led to another novel finding of Nicking endonuclease so far not known in cyanobacteria.

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