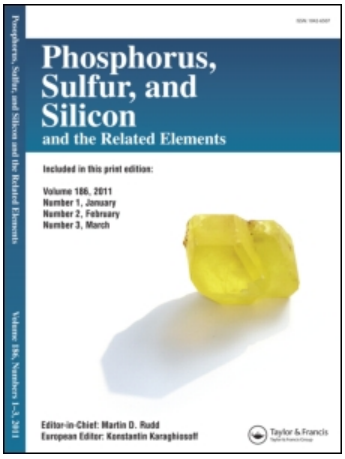

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Kinetic Characterization of Dimeric Maxizyme in the Presence of Cetyltrimethylammonium Bromide

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KINETIC CHARACTERIZATION OF DIMERIC MAXIZYME IN THE PRESENCE OF CETYLTRIMETHYLAMMONIUM BROMIDE

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We constructed a dimeric minizyme that is a hammerhead ribozyme with short oligonucleotide linkers instead of stem-loop II. In a previous study, we demonstrated that this minizyme formed dimeric structure and had higher activity than other minizymes. In order to distinguish monomeric forms of conventional minizymes that have low activity form our dimers with high-level activity, the latter very active short ribozymes were designated 'maxizymes.' Because of their dimeric structure, maxizymes are potentially capable of cleaving a substrate at two different sites simultaneously. We investigated that the maxizyme cleaved tat mRNA at two sites in vitro. Previously, some researchers suggested that the activities of ribozymes in the presence of cethyltrimetylammonium bromide (CTAB) are similar to the activity of ribozyme in vivo. In this study, we carried out the kinetic analyses using some maxizymes in the presence of CTAB. Addition of appropriate amount of CTAB enhanced the activity of a variety of maxizymes. The least active maxizyme was enhanced 100-fold by CTAB. Next, to measure the maxizyme activity in vivo, we transfected maxizyme and target RNA that connected luciferase mRNA as a reporter gene in the HeLa cells. As results, all maxizymes, including the least active maxizyme, inhibited the expressions of target RNAs. Therefore, our maxizymes are useful ribozyme in vivo.

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