Protection against DNase I Degradation

Plasmid DNA encapsulated in chitosan–ATA/pDNA nanoparticles remained intact in the presence of DNase I for up to 4 h of incubation. On the other hand, chitosan/pDNA nanoparticles were completely digested within 4 h of incubation with an equal amount of DNase I. This result demonstrated that conjugated chitosan–ATA can protect encapsulated plasmid DNA from nuclease digestion (Figure 8). This in vitro experiment demonstrates that ATA is indeed capable of inhibiting the nuclease activity in the small intestinal fluid. ATA is known for its inhibitor capacity toward a broad spectrum of nucleases acting as a direct competitive nuclease inhibitor.ATA being bound to the polymer has an inhibitory effect on enzyme activity (Figure 7).

Intestinal Fluid Protection Assay

Plasmid DNA is rapidly degraded by endonucleases of intestinal fluid. The stability of nanoparticles in the intestinal fluid is a key issue in oral gene delivery. To prove that ATA protects the plasmid encapsulation by nucleases, an intestinal fluid protection assay was carried out. Intestinal fluid contains mainly cation-dependent nucleases, such as DNase I, which can be inhibited by ATA. According to Courtois and Torriglia, intestinal fluid contains nucleases that can be divided in three groups: Ca²⁺/Mg²⁺-dependent nucleases, Mg²⁺-dependent nucleases, and cation-independent nucleases. The results from intesti-