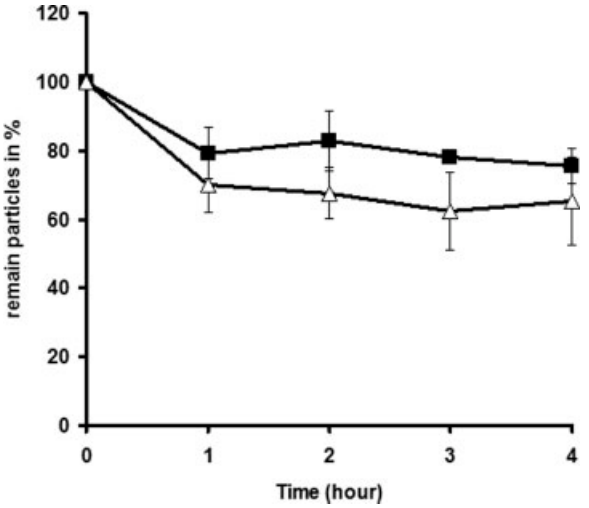

FIGURE 6 Stability of chitosan/pDNA nanoparticles (■) and chitosan–ATA/pDNA nanoparticles (△) in AIF at 37°C. Indicated values are means ± SD of three experiments.

unmodified chitosan. The degradation of the particles can be attributed to the hydrolysis of chitosan substructures being exposed on the surface of particles by lysozyme.[29] It has been reported that lysozyme interacts with the acetamide groups but not with the free amino groups.[18] ATA being bound to the polymer has an inhibitory effect on lysozyme (Figure 7). After incubation with lysozyme for 4 h, chitosan/pDNA nanoparticles were almost completely degraded but not in the case of chitosan–ATA/pDNA nanoparticles (Figure 7).

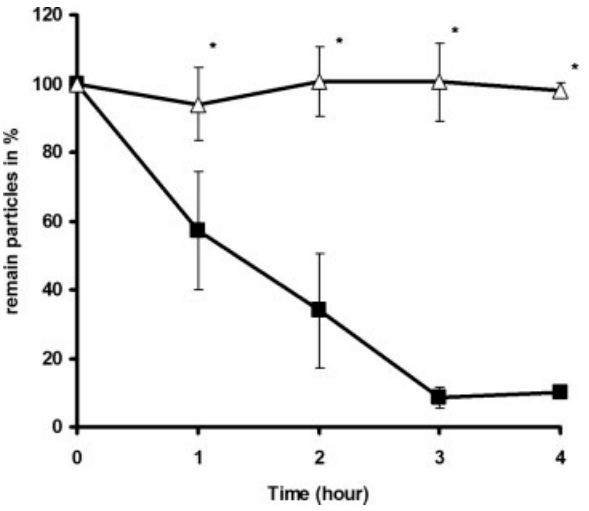

FIGURE 7 Stability of chitosan/pDNA nanoparticles (■) and chitosan–ATA/pDNA nanoparticles (△) in lysozyme (0.1 mg/mL) at 37°C. Indicated values are means ± SD of three experiments. *Differ from chitosan–pDNA nanoparticles, $p < 0.05$.

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.[30] Chitosan–ATA conjugate increased encapsulated DNA stability due to the ATA-mediated inhibition of nuclease activity.

Intestinal Fluid Protection Assay

Plasmid DNA is rapidly degraded by endonucleases of intestinal fluid.[31] The stability of nanoparticles in the intestinal fluid however is a key issue in oral gene delivery. To confirm that ATA protects the plasmid from degradation 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.[32] According to Coulis and Torriglia,[33] intestinal fluid contains nucleases that can be divided in three groups: Ca^{2+}/Mg^{2+}-dependent nucleases, Mg^{2+}-dependent nucleases, and cation-independent nucleases. The results from intes-

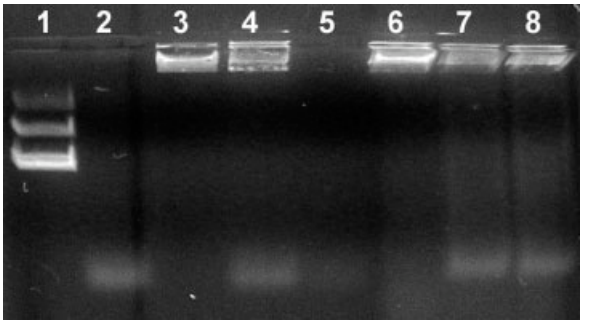

FIGURE 8 Agarose gel electrophoresis for DNase I protection assay. DNA and nanoparticles were incubated with 1 U/mL of DNase I for 1, 2, and 4 h at 37°C. Lane 1, untreated control DNA; lane 2, DNA after incubation for 1 h; lane 3, untreated control chitosan/pDNA nanoparticles; lanes 4 and 5, chitosan/pDNA nanoparticles after 2- and 4-h incubation with DNase I; lane 6, untreated control chitosan–ATA/pDNA nanoparticles; lanes 7 and 8, chitosan–ATA/pDNA nanoparticles after 2- and 4-h incubation with DNase I.