Abstract

Two novel methods for the covalent immobilization of recombinant thermostable α-amylase enzyme has been introduced by this study. Thermostable α-amylase was successfully expressed in *E. coli* and further purified. Thermostable α-amylase was immobilized on the surface of magnetic nanoparticles coated with silica by the carbodiimide activation and by 3-APTES surface modification. Immobilized enzyme was further characterized and the stability and activity of free and immobilized enzyme was evaluated. FT-IR analysis confirmed the immobilization of enzyme with magnetic nanoparticles. Low enzyme loading (6 mg/ml) was responsible for the efficient activity of enzyme after immobilization by carbodiimide activation. Immobilized enzyme showed significant activity at neutral and acidic pH. In addition, better resistance of α-amylase to the inhibitory effect of metal ions and inhibitors was observed after immobilization. Immobilization with magnetic nanoparticles ensured fast and efficient recovery. Enzyme showed increased activity even at higher temperature of 100°C after immobilization. The reusability factor of immobilized α-amylase was also evaluated and enzyme retained 50% of its activity after 30 consecutive operations at 90°C. Enzyme when immobilized with magnetic nanoparticles by 3-APTES modification showed increased stability and activity at wide range of temperature and pH. Immobilized α-amylase remained stable even at acidic and basic pH along with neutral pH as compared to free enzyme. α-amylase enzyme of concentration 2.5 mg/ml was required for the immobilization with the incubation time of 3 hours. Gluteraldehyde, when used as a spacer arm, ensured higher efficiency of enzyme immobilization along with the fast recovery as compared to the prior method used. Immobilized enzyme showed thermally and chemically more stable activity as compared to the free enzyme. Enzyme when immobilized with nanoparticles retained its 50% original activity after 35 consecutive operations at 90°C and 16 consecutive activity cycles at 100°C. The results indicated better stability and activity of immobilized α-amylase in the presence of metal ions and inhibitors as compared to free enzyme.