Determination of BHET and MHET Hydrolase Activities

Enzymatic activity was assayed using BHET (bis(2-hydroxyethyl) terephthalate), a soluble fragment of PET, as the model substrate. A BHET stock solution was prepared by dissolving 20 mg BHET in 10 mL dimethyl sulfoxide (DMSO). The reaction was performed at 30 °C for 30 min in a total volume of 0.6 mL containing 50 mM Na_2HPO_4-HCl buffer (pH 7.5), 100 mM NaCl, and 5 mM BHET. The reaction was initiated by the addition of 50 nM purified enzyme. To determine the optimum pH, different buffers covering a pH range of 5.0–10.0 were used: 50 mM citrate-sodium citrate buffer (pH 5.0–6.5), 50 mM Na_2HPO_4-NaH_2PO_4 buffer (pH 6.0–8.0), 50 mM Tris-HCl buffer (pH 8.0–9.0), and 50 mM glycine-NaOH buffer (pH 9.0–10.0). Reactions were terminated by adding methanol to one-third of the reaction volume, and degradation products were analyzed by high-performance liquid chromatography (HPLC).

The determination of MHET (mono(2-hydroxyethyl) terephthalate) hydrolase activity was performed using a similar protocol, except that the reaction mixture was scaled to 4 mL and contained 500 nM purified enzyme. Aliquots of 0.6 mL were collected at specific time intervals, quenched with one-third volume of methanol, and analyzed by HPLC.

All enzymatic assays were carried out in triplicate, and results are reported as mean values ± standard error of the mean (SEM).