THE EFFECTS OF MICROGRAVITY ON THERMOSTABLE T1 LIPASE PROTEIN CRYSTAL
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The quest for the characterizations of intrinsically thermostable T1 lipase either physicochemically or structurally is a prominent task. T1 lipase can be effectively used as an additive in detergent formulations, and as a biocatalyst for natural oil-based pharmaceuticals, foods and fine chemicals. The thermoalkaliphilic T1 lipase gene of *Geobacillus zalihae* sp. nov. strain T1 (Rahman et al., 2007) was overexpressed in the pGEX vector in *E. coli* (Loew et al., 2004). Expression of T1 lipase as a glutathione S-transferase (GST) fusion protein in prokaryotic systems was expected to allow rapid purification of recombinant T1 lipase through affinity chromatography. High-yield purification of T1 lipase was achieved through two-step affinity chromatography with a final specific activity and yield of 958.2 U/mg and 51.5%, respectively. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis showed that the purified T1 lipase appeared as 39 kDa after the removal of the 26 kDa GST tag from the digested fusion lipase. However, the native molecular weight of T1 lipase was determined to be approximately 43 kDa by gel filtration chromatography. The size was similar to its predicted molecular weight, but slightly bigger than its denatured form obtained through SDS-PAGE (Leow et al., 2007).

The T1 lipase was extensively characterized, both physicochemically and spectroscopically using Circular Dichroism (CD) and spectrofluorometry. Although mature T1 lipase is most active in the temperature range of 65-75°C, it has an optimum temperature of 70°C. T1 lipase was intrinsically stable at 60 and 65°C, as revealed by half-lives of 12 and 5 h 15 min, respectively. At 70°C, its half-life was 1 h 10 min and T1 was able to retain more than 20% of its lipase activity at the same temperature even up to 10 h of treatment time. This is almost concurrent with the denatured protein analysis using circular dichroism (CD) which revealed thermal denaturation (Tm) for T1 lipase to be around 72.2°C. The purified T1 lipase displayed a broad pH activity of pH 6.0-11.0, with an optimum pH of 9.0 in 50 mM glycine-NaOH buffer, when olive oil was used as substrate. Despite the high activities within a broad pH range, almost none or low T1 lipase activity was observed at pH below 6.0 and above 11.0 (Leow et al., 2007).

High temperature crystallization of T1 lipase was a new discovery. From the literature, there was no record of high temperature crystallization especially for thermostable lipases. To date, only two thermostable lipases from *Bacillus stearothermophilus* L1 and *Bacillus stearothermophilus* P1 have been successfully crystallized at 23 and 16°C, respectively (Jeong et al., 2001; Sinchaikul et al., 2002).

Temperature is a noninvasive control parameter for protein crystallization, as compared to other parameters such as protein concentration, precipitants, buffer and pH. Temperature can be used as an alternative route in protein crystallization to precisely control the nucleation and post nucleation crystal growth without manipulation of solute concentrations. An attempt was made to crystallize T1 mature lipase at temperatures ranging from 16 to 70°C to investigate the crystal growth range and sizes. Crystallization of T1 mature lipase was still possible up to 60°C but no crystal formation was observed at 70°C as denaturation occurred prior to crystallization (Leow et al., 2007). The T1 mature lipase showed denaturation at 70°C after 24 h without forming any crystal since its half-life was 70 min at 70°C. Heat inactivation tends to unfold the T1 mature lipase, and the resulting protein may lose the conformational stability of the folded state.

The atomic details of T1 lipase solved at 1.5 Å unveiled a novel cation-π interaction (Matsumura et al., 2008) which was the first report of such a ligand among thermostable lipases (Figure 1a). A mutant F16L has revealed F16 was a key residue in this interaction as shown in Figure 1b.

![Figure 1](image-url)

**Figure 1.** a) Electron density map of native T1 lipase crystal. Potassium ion denoted as M showed an interaction with the phenylalanine F16 residue. S113 and H358 are part of T1 lipase active site. b) Electron density map of mutant F16L exhibiting no cation-π interaction due to the loss of the phenylalanine aromatic ring.
Space crystallization of T1 lipase was carried out using the high-density protein crystal growth apparatus (HDPCG) developed by the University of Alabama and BioServe Space Technologies USA, utilizing the vapor diffusion method. The Soyuz FG rocket carrying the Soyuz TMA-11 spacecraft lifted off from the launching pad at Site 1 at Baikonur Cosmodrome in Kazakhstan on October 10, 2007, at 17:22:14 Moscow summer time. The crew included our first Angkasawan, Dr. Sheikh Muszaphar Shukor Al Masrie who carried out the activation and deactivation processes in ISS. After 11 days Dr. Sheikh Muszaphar Shukor Al Masrie, returned to the Earth on October 21, 2007, aboard Soyuz TMA-10. X-ray diffraction of space and ground crystals data were collected at SPring-8 BL41XU synchrotron radiation facility, Hyogo, Japan. Microgravity apparently improved the size and interface of crystals significantly. As shown in Figure 2a, crystals grown in the microgravity environment were much larger than earth-grown crystals (Figure 2c). This is a common finding where the zero gravity effect has proven to promote better crystal internal packing. The microgravity effect on crystallization of T1 lipase was clearly evidenced by the finer atomic details at 1.35Å compared to 1.8Å obtained on earth. Marked improvement of the electron density map from space grown crystals can be observed as compared to the poorer resolution of earth grown crystals. Such examples can be seen in the bulky side chain residues like His in space (Figure 2b)- and earth (Figure 2d)-grown crystals, respectively. This finding resembles the improved electron density maps of ring side chains demonstrated by Sjölin et al. (1991) in the case of ribonuclease. It is concluded that structural elucidation following high-temperature crystallization and in microgravity helps in the understanding of protein structure in general and in the unfolding process in particular.

![Figure 2](image_url)

**Figure 2.** a) Space grown T1 lipase crystal after 8 days of activation on board International Space Station (ISS) b) High quality electron density map of histidine residue (H14) in T1 lipase structure c) Earth grown T1 lipase crystal. Note the size and the quality of the crystals. d) Histidine residue (H14) from an earth-grown crystal. Note the density map quality colored as blue.

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**REFERENCES**


