Supplemental Information

Purification of poliovirus polymerase

Eight 750 mL cultures of BL21-pLysS DE3 E.coli containing the T7-based pT5T3D expression plasmid were grown in 2xYT media with 500 µg/mL ampicillin, 30 mM potassium phosphate (pH 7.7), 17 µg/mL chloramphenicol, and 5 mM glucose to an OD600 of 0.2 at 37°C. The cultures were then shifted to 25°C and grown to an OD600 of 0.4. IPTG was added to 500 µM and the cultures were incubated further for 16-18 hours at 25°C. The cells were harvested at 3300xg for 15 min. at 4°C and frozen at -20°C. Upon thawing, each cell pellet was resuspended in 400 mL of 100 mM potassium phosphate (pH 7.5), 2 mM DTT, 0.1 mM EDTA, and 0.02% sodium azide. Cells were disrupted by sonication with a Misonix (Farmingdale NY) XL2020 sonicator using the 0.5 inch probe tip at setting 5 for fifteen 30 sec. intervals. The lysate was centrifuged at 16,000×g for 30 min at 4°C. The supernatant was retrieved and ammonium sulfate was added to a final concentration of 22.6% (w/v) and allowed to mix at 4°C for 30 min. The precipitate was collected at 16,000×g for 30 min at 4°C, resuspended in 40 mL of 25 mM Hepes (pH 7.5), 50 mM NaCl, 2 mM DTT, 0.1 mM EDTA, and 0.02% sodium azide, and dialyzed overnight at 4°C in 40 mm diameter, 25,000 MWC dialysis tubing (Spectra/POR, Los Angeles CA) against 8 L of the same buffer. The dialyzate was centrifuged at 25,000xg for 30 min at 4°C. The polymerase was subject to further purification by FPLC using 20 mL Pharmacia HP S-sepharose and HP Q-sepharose columns sequentially. All column buffers contained 15% glycerol, 0.5% n-octyl glucoside, 2 mM DTT, 0.1 mM EDTA and 0.02% sodium azide. Buffers for the S-sepharose column contained 50 mM bicine (pH 8.5). Buffers for the Q-sepharose column contained 25 mM Tris (pH 8.0). The columns were washed with two column volumes of buffer containing 1 M NaCl and equilibrated with 2 column volumes of buffer containing 100 mM NaCl at 2 mL/min prior to use. The supernatant was loaded on the S-sepharose column at 1 mL/min and the column was washed with column buffer containing 100 mM NaCl at 1 mL/min until the baseline stabilized (3 column volumes). A linear gradient from 100mM to 415 mM NaCl was used to elute the polymerase from the column. The polymerase eluted in a broad peak from 240 to 300 mM NaCl. Pooled fractions containing polymerase were diluted with Q-sepharose column buffer to 100 mM NaCl and loaded onto the Q-sepharose column, which was washed as described above. The polymerase eluted from the Q-sepharose column at approximately 280 mM NaCl. Pooled fractions containing the polymerase were mixed with cold glycerol to 50% final concentration, concentrated using Vivaspin 20 concentrators (Vivascience), and aliquots stored at -80°C.