Citrates lyase activity exists in eukaryotes, bacteria, and archaea and plays a central role in cellular energy metabolism. There are two types of citrate lyases, ATP-dependent and ATP-independent. The latter, which exists only in bacteria, converts citrate to acetate and oxaloacetate using a two-step reaction catalyzed by a stable heterotrimeric protein complex comprising alpha-beta-gamma subunits. We selected three bacterial genomes in which the alpha-beta-gamma subunits of citrate lyase are all encoded on the chromosome in a continuous, co-cistronic geometry. The genes encoding the entire complex were subsequently amplified together using a single PCR reaction on genomic DNA. This approach enabled the entire citrate lyase complex from three organisms to be purified to homogeneity using a single step of Ni-NTA chromatography followed by gel-filtration chromatography. One of the three citrate lyase complexes purified yielded crystals diffracting to 4 Å. Six-fold non-crystallographic symmetry averaging enabled a high quality structure to be determined for the 18-subunit alpha-beta-gamma citrate lyase complex. This structure provides insight into the multistep catalytic mechanism employed by this enzyme.

**Keywords:** citrate lyase complex