Results

Structure Determinations

Two different *Saccharomyces cerevisiae* Vps4 constructs that spanned residues 104-437 and 122-437 were expressed in *E. coli,* crystallized, and structurally characterized by X-ray crystallography using the human VPS4B structure as an initial search model[25]. Both constructs lacked N-terminal MIT domains and segments of the ensuing linker (see Fig. 1), and both also contained the E233Q mutation, which allows ATP binding but blocks ATP hydrolysis[16]. $Vps4_{122-437}$ crystallized in space group $P6_522$, with one protein molecule per asymmetric unit and a sulfate ion bound at the active site. This structure was refined at a resolution of 2.7 Å and an R_{free} value of 28.7% (Table 1). $Vps4_{104-437}$ crystallized in space group $P2_12_12_1$ with three molecules per asymmetric unit and was refined at a resolution of 3.25 Å to an R_{free} value of 28.9%. Molecules A and B showed good density for ATPγS at the active site (Fig. 2), whereas molecule C also had active site electron density but clearly lacked density for the adenine nucleoside. The density could be adequately modeled as an ethylene glycol molecule in complex with a magnesium ion, and this is therefore our tentative assignment for the bound ligand in this case. Crystallographic statistics are summarized in Table 1, and both structures are generally well ordered, albeit at modest resolution. As detailed in Table 2, the ordered regions of all constructs begin at approximately residue 122, and we therefore refer to the proteins interchangeably as $Vps4_{\Delta MIT}$, with their bound ligands designated as "SO_4" (crystal form 1), "ATPγS" (molecules A and B in crystal form 2), and "ethylene glycol-Mg^{2+}" (molecule C in crystal form 2).