

Differences in Substrate Specificity of Five Bacterial Wax Ester Synthases

Supplemental Materials

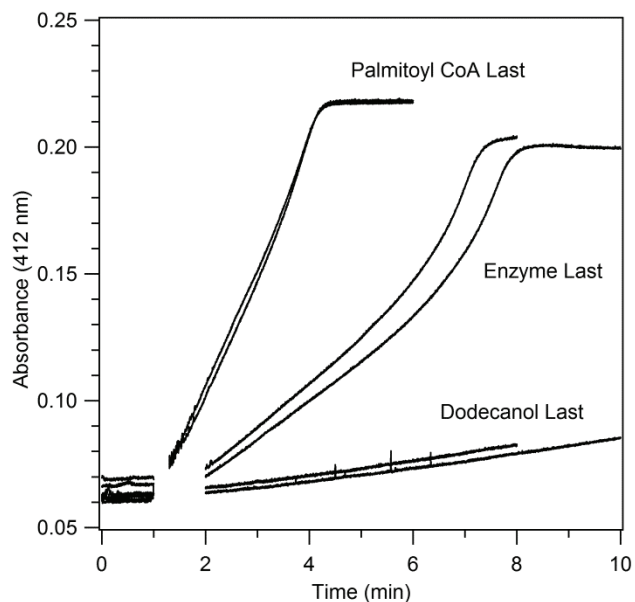


Figure S1. Absorbance time course traces obtained from order of addition experiments. Each sample minus the component indicated was mixed and incubated for at least one minute prior to addition of the final component in the spectrophotometric assay to confirm a stable baseline result (described in methods), and the reaction was then initiated with the remaining component (labeled on figure). For each condition, duplicates were run and are shown. Some data points between 1 and 2 minutes during the mixing of each sample were omitted for clarity. This figure shows the data obtained with Ac1. A summary of the data from each protein is shown in Table 2.

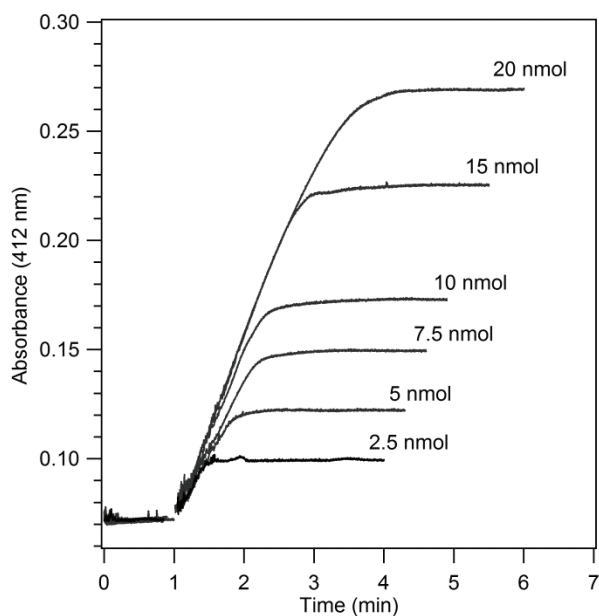


Figure S2. Absorbance time course traces of palmitoyl-CoA activity at various concentrations. Shown are traces obtained from spectrophotometric assays using 20 nmol of dodecanol and various quantities of palmitoyl-CoA (labeled on figure) in a cuvette containing approximately 1.2 mL of total solution. Each sample was mixed and monitored for at least one minute prior to addition of the palmitoyl-CoA to initiate the reaction (described in methods), and then the reaction was followed until a plateau was found in the data indicating completion of the reaction. Portions of the data points between 1 and 2 minutes were omitted for clarity, as this occurred during the mixing of the sample. This figure shows the data obtained with Ma1, and illustrates that the initial rates were similar for each concentration of palmitoyl-CoA added, but the reaction rate dropped off substantially as the substrate ran out.

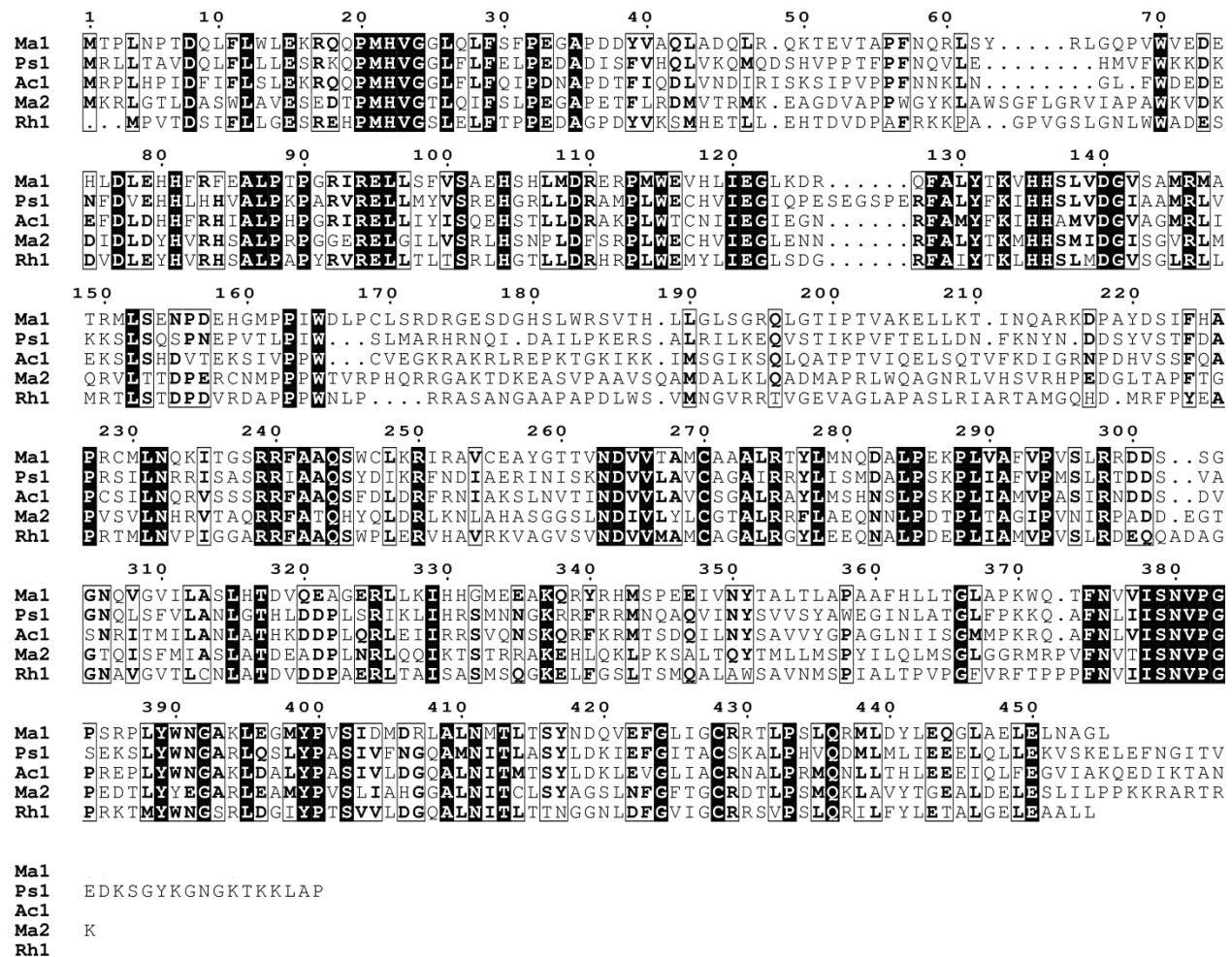


Figure S3. Multiple alignment and consensus sequence comparison of the amino acids for the proteins utilized in these studies. Each protein was fused such that the N-terminal methionine residue (shown) was removed and the gene fused in frame with the N-terminal maltose binding protein and a C-terminal 8X His-Tag was fused in frame prior to the stop codon. Numbering is for Ma1. Alignments were generated as described in the materials and methods.

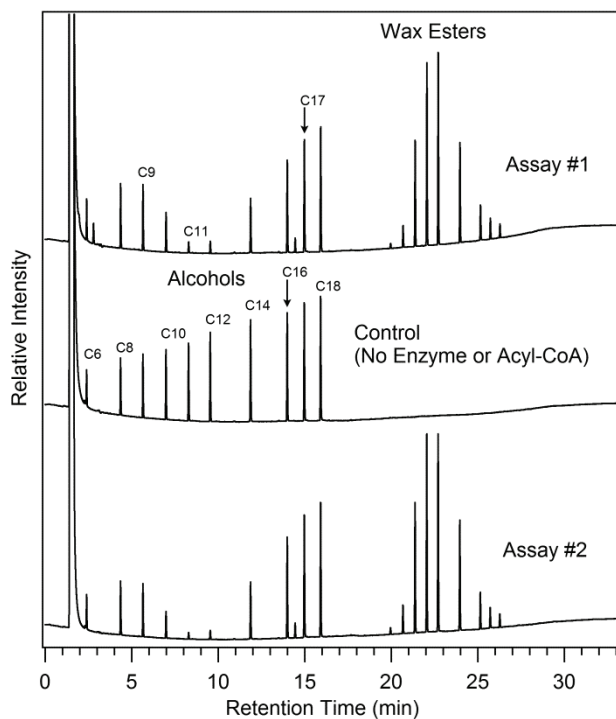


Figure S4. Representative gas chromatograms for substrate selectivity assays used for comparison of different WS/DGAT enzymes. Shown are the results obtained using a GC/FID analysis obtained with Ma1 assayed in the presence of a range of fatty alcohols. A control sample of only the fatty alcohols used in the assays is included (middle) to illustrate the separation of the fatty alcohol substrates and the wax ester products (between 19 and 27 minutes). The results of two separate assays (top and bottom) utilized to prepare the data comparisons in Figure 5 are shown to illustrate reproducibility and precision of the assay. Various components (alcohols and resulting wax esters) are labeled on the figure, and the wax ester products were further confirmed by GC/MS, as described in the materials and methods.