UNIVERSIDAD DE BARCELONA

Facultad de Farmacia

Departamento de Bioquímica y Biología Molecular

REDESIGN OF CARNITINE ACETYLTRANSFERASE SPECIFICITY BY PROTEIN ENGINEERING

ANTONIO FELIPE GARCIA CORDENTE

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CONCLUSIONS

1. The use of *Saccharomyces cerevisiae* as an expression system is a valid model in which to study structure-function relationships in CrAT and COT.

2. The amino acids Ser^{119}, Phe^{138}, Gly^{249} and Ala^{515} in CrAT, identified by bioinformatics methods, are not involved in acyl-CoA selectivity in that enzyme.

3. The point mutation of Met^{564} to Gly in CrAT broadens the specificity of the enzyme toward medium-chain acyl-CoAs, converting rat CrAT into a pseudo-COT.

4. Mutation of the orthologous glycine (Gly^{553}) to methionine in COT decreases activity towards its natural substrates, medium-chain acyl-CoAs, and increases activity toward short-chain acyl-CoAs. Gly^{553} is responsible for the substrate specificity of COT.

5. Our three-dimensional models show that the side chain of Met^{564} acts as a lid, impeding entry of medium-chain acyl-CoAs, and when this voluminous side chain is removed in the CrAT mutant M564G, a hydrophobic pocket is made accessible, extending the sensitivity of the enzyme to long-chain acyl-CoA substrates.

6. CrAT Asp^{356} is also involved in determining acyl-CoA specificity in CrAT. Its mutation to Ala, along with the mutation of Met^{564} to Gly, creates an artificial enzyme that behaves as a CPT in terms of acyl-CoA specificity.

7. Our kinetic data show that His^{343} and Glu^{347} are critical for catalysis in CrAT.

8. Structure-based mutagenesis in COT indicates that selectivity for long-chain substrates is determined by more complex factors than specific amino acid variations between the different members of the family.

9. Electrostatic and steric factors contribute similarly to the discrimination between choline and carnitine in CrAT. Mutation of four amino acids in CrAT (A106M/T465V/T467N/R518N) shifts the catalytic discrimination between L-carnitine and choline in favour of the latter substrate.

10. C75-CoA is a potent inhibitor of CrAT mutant M564G and D356A/M564G, but does not inhibit wt CrAT, indicating that the enzyme is only sensitive to the inhibitor when the hydrophobic pocket of CrAT is accessible to longer acyl-CoAs.

11. Overexpression of *CAT2* produces a modest although significant reduction in the levels of some volatile esters in fermenting yeast.