Insights into Binding Specificity of Human Heart-Type Fatty-Acid Binding Protein

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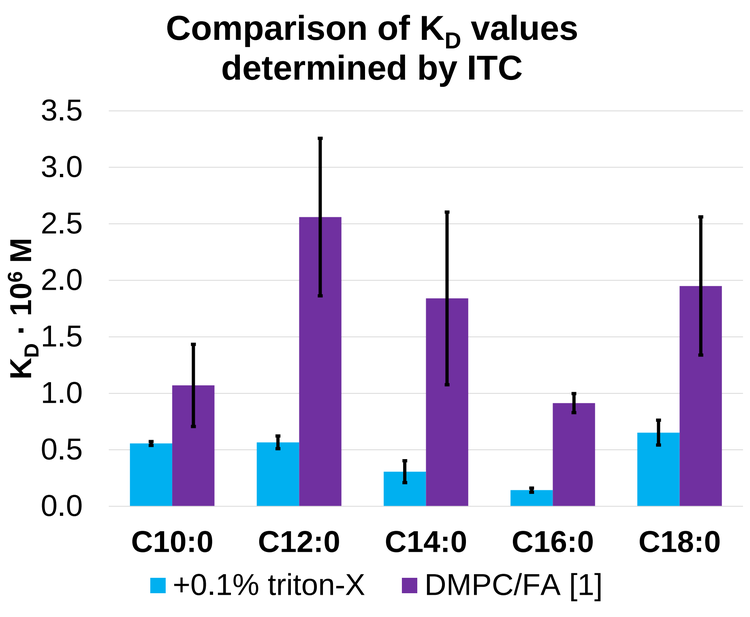
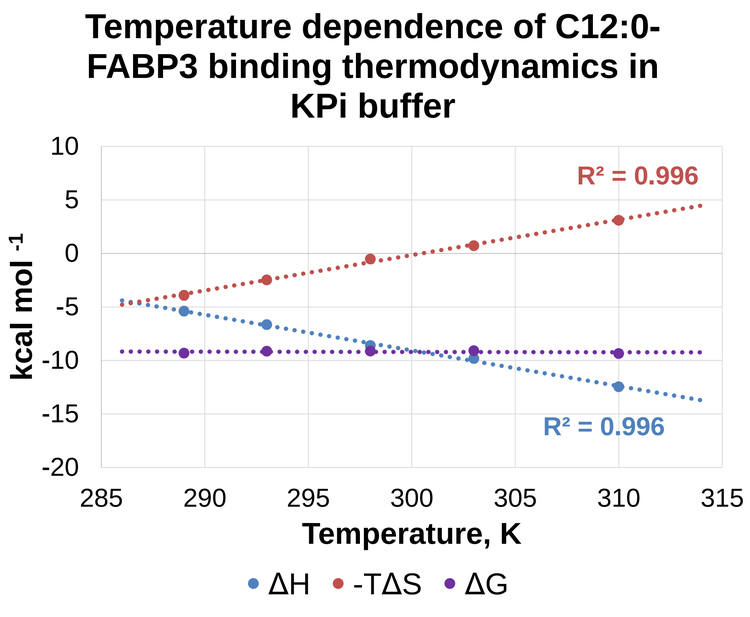
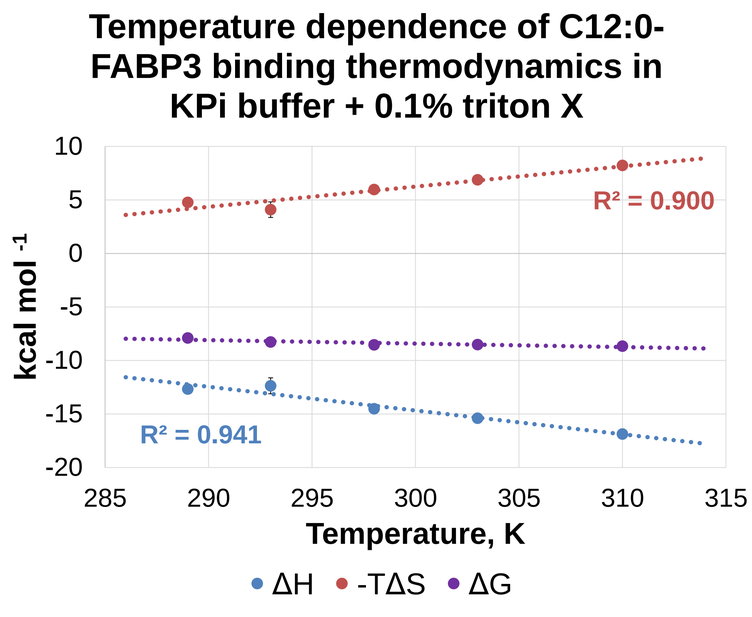
Introduction

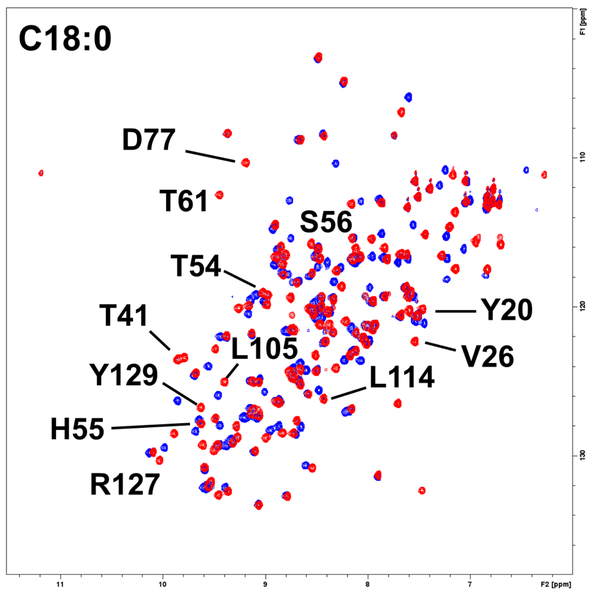
Fatty-acid binding proteins (FABPs) belong to a small family of a cytosolic transport proteins whose main function is related to binding and relocation of highly hydrophobic long-chain fatty acids (FA). Currently, eight tissue-specific types of FABP are known: liver, intestinal, heart, adipocyte, epidermal, ileal, brain, and testis. It is proposed that FABPs also protects tissues from the high toxicity of FA and their metabolic intermediates.

In this work, we focused on binding characterization of FA and FA-carnitine esters to human heart-type-FABP (H-FABP or FABP3) by means of isothermal titration calorimetry (ITC) and NMR.

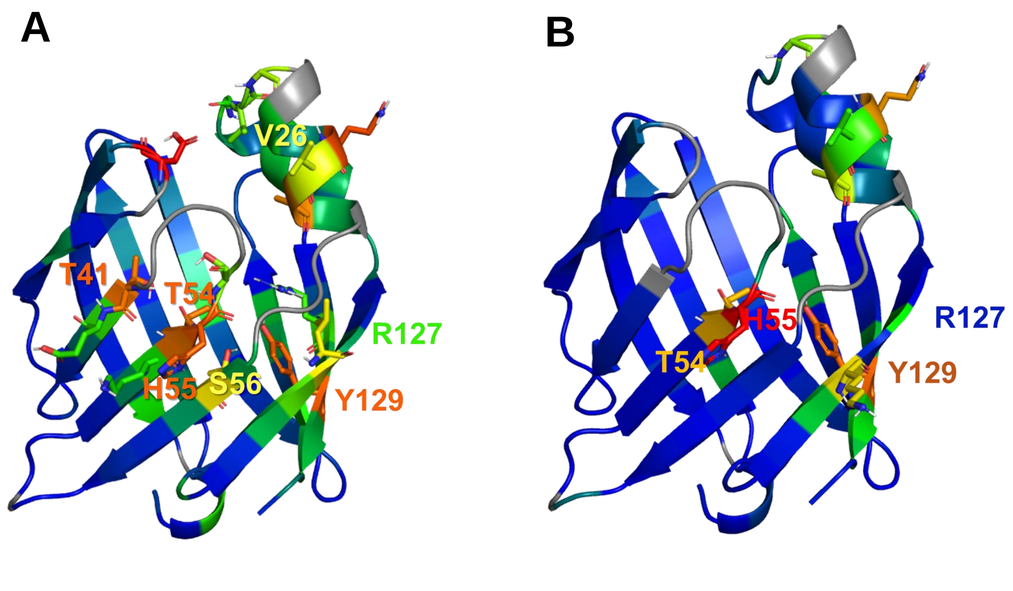




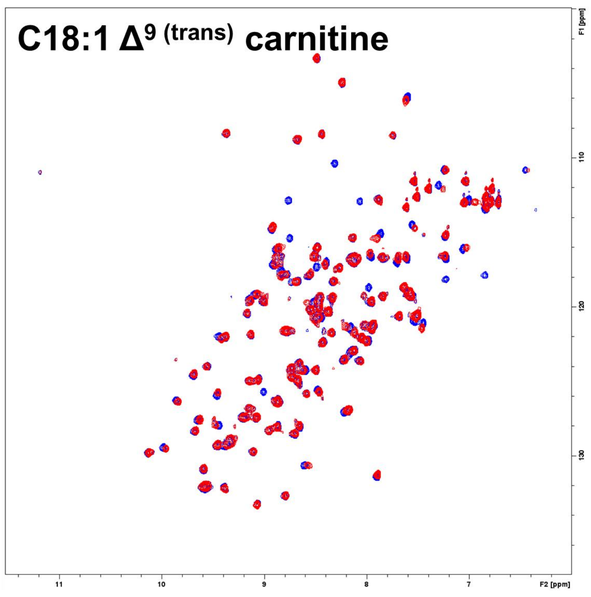




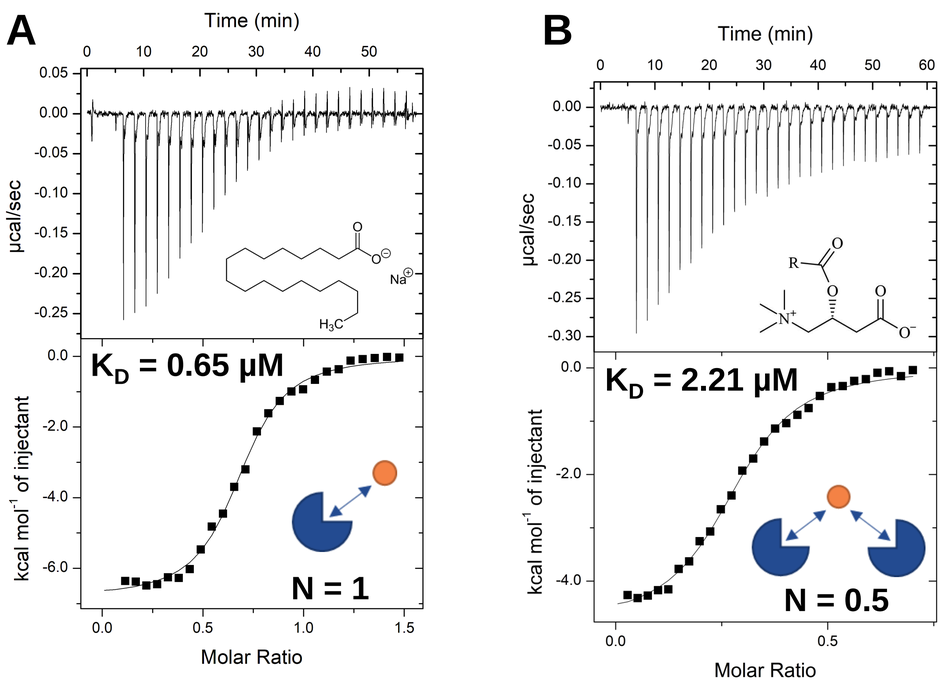
2D ^{1}H-^{15}N-HSQC spectra of ^{15}N-labelled FABP3: apo-form (blue) and in complex (red) with stearate (C18:0)



Crystal structure of FABP3 (PDB ID: 4TKJ) coloured in gradient from blue (min) to red (max) based on chemical shift perturbation upon binding of stearate (A) or carnitine elaidate (B) to FABP3



2D ^{1}H-^{15}N-HSQC spectra of ^{15}N-labelled FABP3: apo-form (blue) and in complex (red) with carnitine elaidate (C18:1 Δ^{9})



Isothermal titration calorimetry (ITC) data of stearate (A) and carnitine elaidate (B) binding to recombinant FABP3 delipidated by 70%

Conclusions

- ITC assay was developed and successfully applied to characterize FABP3 binding with long-chain FA.
- 0.1% triton-X additive did not change binding affinity, $\Delta G/K_D$, but slightly strengthened enthalpic, ΔH, and weakened entropic, $-T\Delta S$, contribution.
- Recombinant fatty-acid free FABP3 is able to bind not only long-chain FA but also unsaturated FA-carnitine esters.
- Combination of ITC and NMR data reveal that unsaturated FA-carnitine esters have different binding mechanisms in comparison to FA with stoichiometry close to 0.5.

Acknowledgement

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