Acta Crystallographica Section D

Volume 70 (2014)

Supporting information for article:

Insights into the binding specificity and catalytic mechanism of *N*-acetylhexosamine 1-phosphate kinases through multiple-reaction complexes

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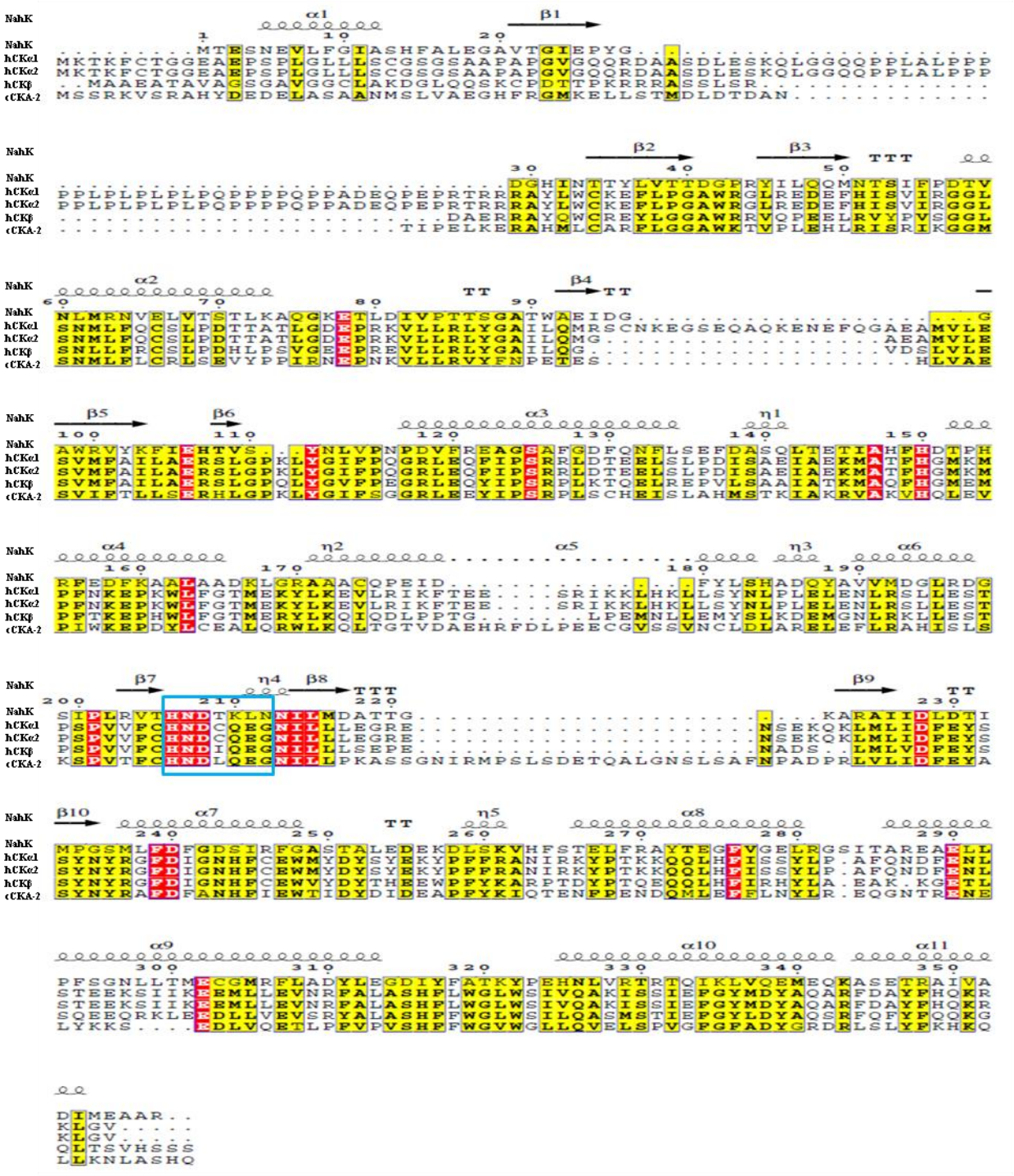
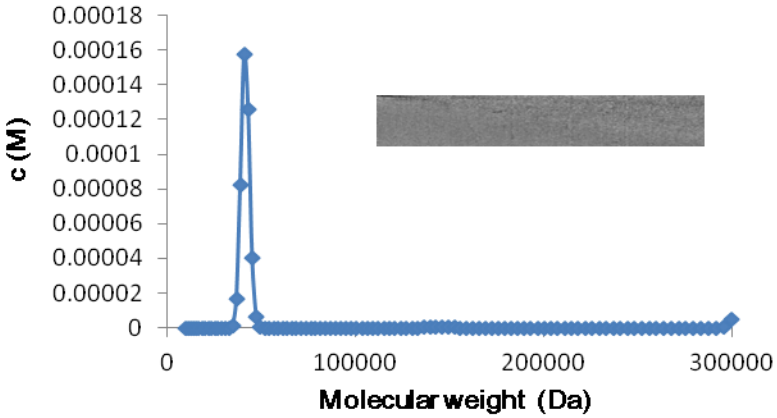


Figure S1 Sequence alignment for NahK and choline kinases. The secondary structure of NahK (JCM1217) is placed on the top of the alignment. Identical residues are colored red, and similar residues are shown in yellow. The conversed Brenner's motif is labeled in cyan. NCBI accession numbers are BAF_73925, NP_001268, NP_997634, NP_005189 and NP_001024480 for NahK, hCKα1, hCKα2, hCKβ and cCKA-2, respectively, wherein hCK and cCK denote human and *C. elegans* choline kinase, respectively.

(a)



(b)

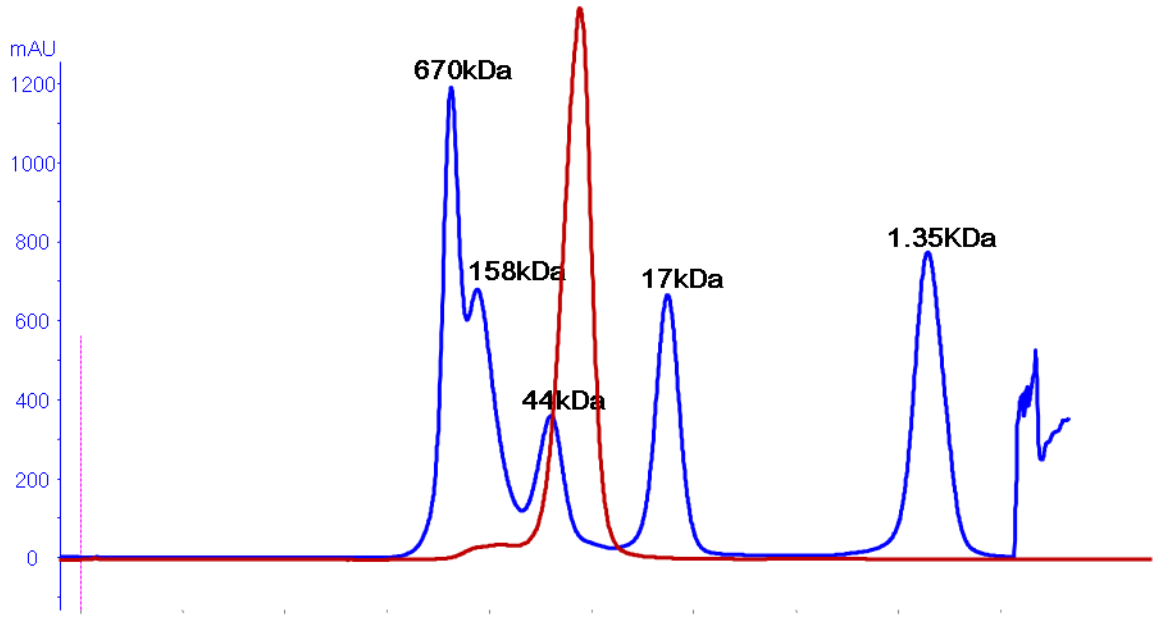


Figure S2 Analytical ultracentrifugation (AUC) and gel filtration chromatography analysis for NahK. (a) The molecular weight of NahK was determined by the sedimentation coefficient (*s*). The gray bar is the residual bitmap. (b) The FPLC chromatographs of NahK and protein standards (Bio-rad), which are shown as brown and blue lines, respectively.

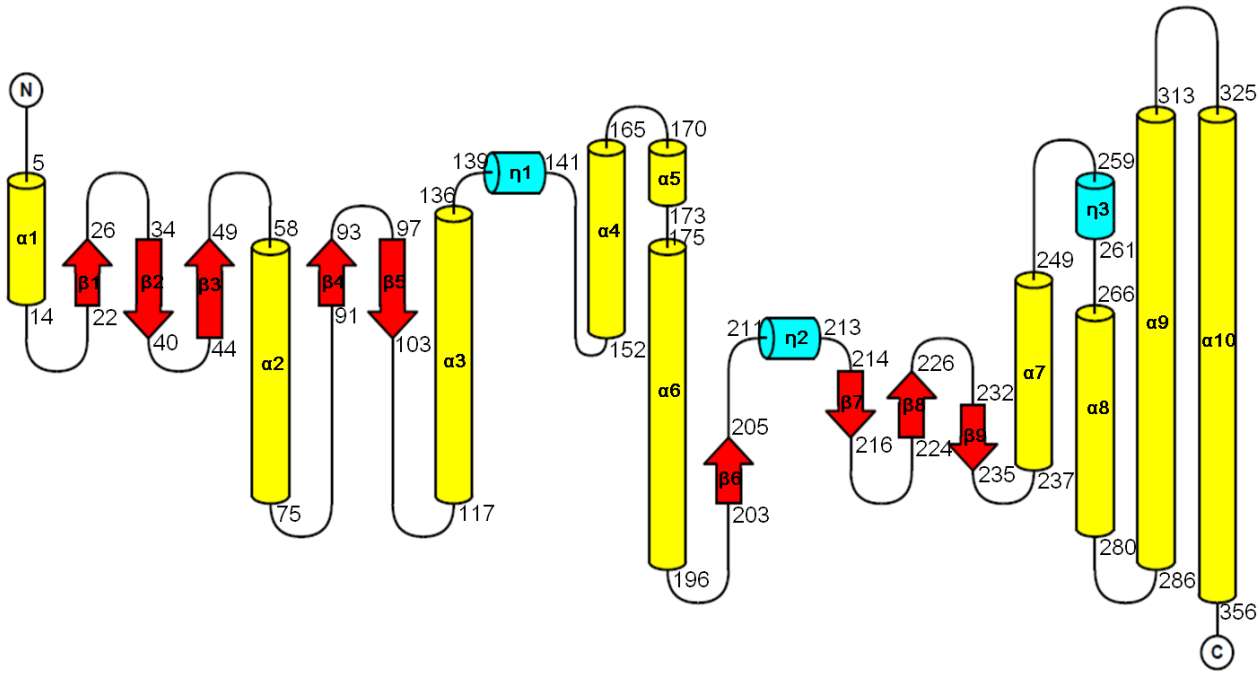


Figure S3 The topology of NahK.

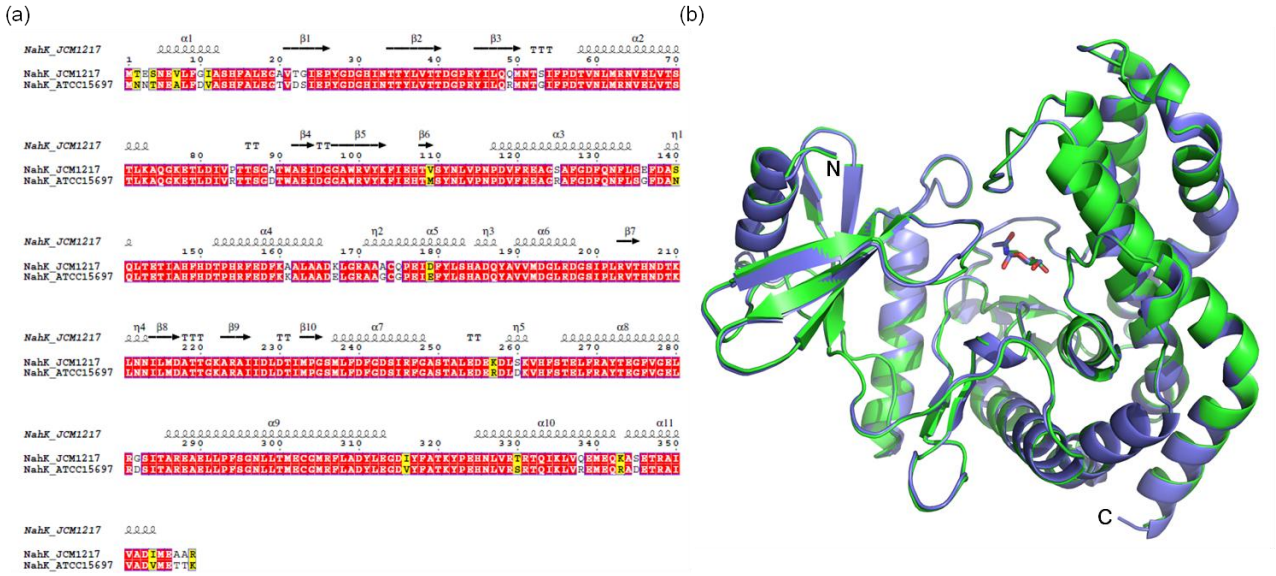


Figure S4 (a) Sequence alignment for NahK_JCM1217 and NahK_ATCC15697. The secondary structure of NahK_JCM1217 is placed on the top of the alignment. Identical residues are shown in red, and similar residues are shown in yellow. (b) Superimposition of NahK_JCM1217 (green) and NahK_ACTT15697 (purple) (RMSD of 0.37 Å over 352 Cα atoms). NahK and GlcNAc are shown as ribbon diagrams and stick models, respectively.

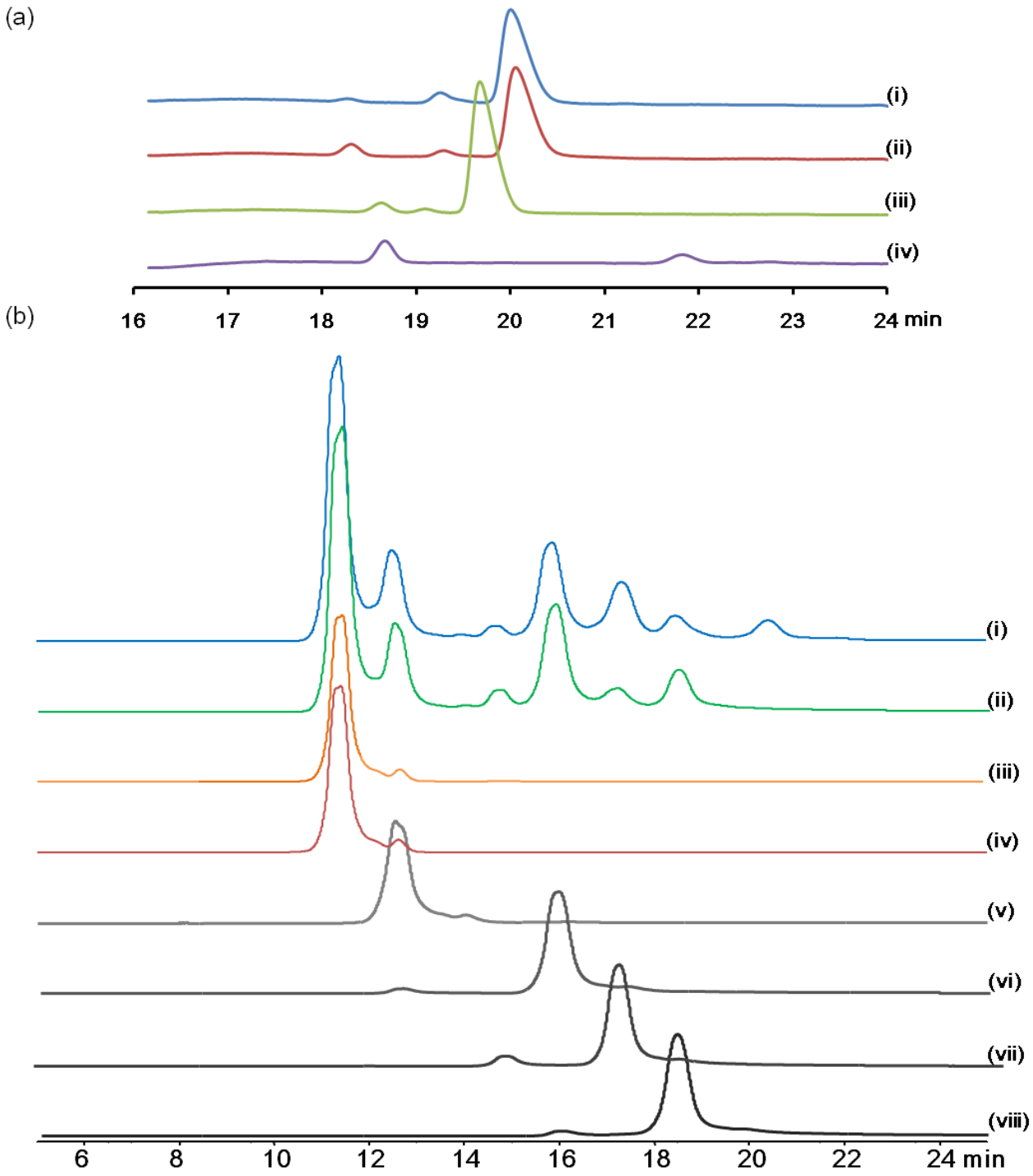


Figure S5 LC traces. (a) HPAEC-PAD traces of sugar-1Ps: (i) the GlcNAc-1P standard; (ii-iv) GlcNAc-1P, GalNAc-1P and mannose-1P (ii-iv are reaction products). (b) HPLC traces of UDP-sugars: (i) the formation of UDP-GlcNAc in the presence of NahK, GlmU, ATP, UTP, and GlcNAc; (ii) the formation of UDP-GalNAc in the presence of NahK, GlmU, ATP, UTP, and GalNAc; (iii-viii) the standards of UDP-GlcNAc, UDP-GalNAc, AMP, ADP, UTP and ATP, respectively.