SUPPLEMENTARY MATERIAL

Structures of enzyme intermediate complexes of yeast Nit2: insights into the catalytic mechanism and different substrate specificity compared to mammalian Nit2*

Hejun Liu[1,2], Yongxiang Gao[1,2], Mengying Zhang[1,2], Xiaoting Qiu[1,2], Arthur J.L. Cooper[3], Liwen Niu[1,2] and Maikun Teng[1,2]

[1]Hefei National Laboratory for Physical Sciences at the Microscale and School of Life Sciences, University of Science and Technology of China, Hefei, Anhui, 230026, People's Republic of China, [2]Key Laboratory of Structural Biology, Chinese Academy of Sciences, Hefei, Anhui, 230026, People's Republic of China, and [3]Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, NY 10595, USA

Table of Contents

Name	Title
Fig. S1	12% SDS-PAGE analysis of Nit2 proteins
Fig. S2	Structure of WT yNit2
Fig. S3	Chromatography of standard proteins and yNit2.
Fig. S4	Putative structure of the ligand bound to the mutant enzyme yNit2-C169S
Table S1	Amino acid sequences of yNit2, recombinant yNit2, hNit2 and recombinant C169S and R250Y mutants

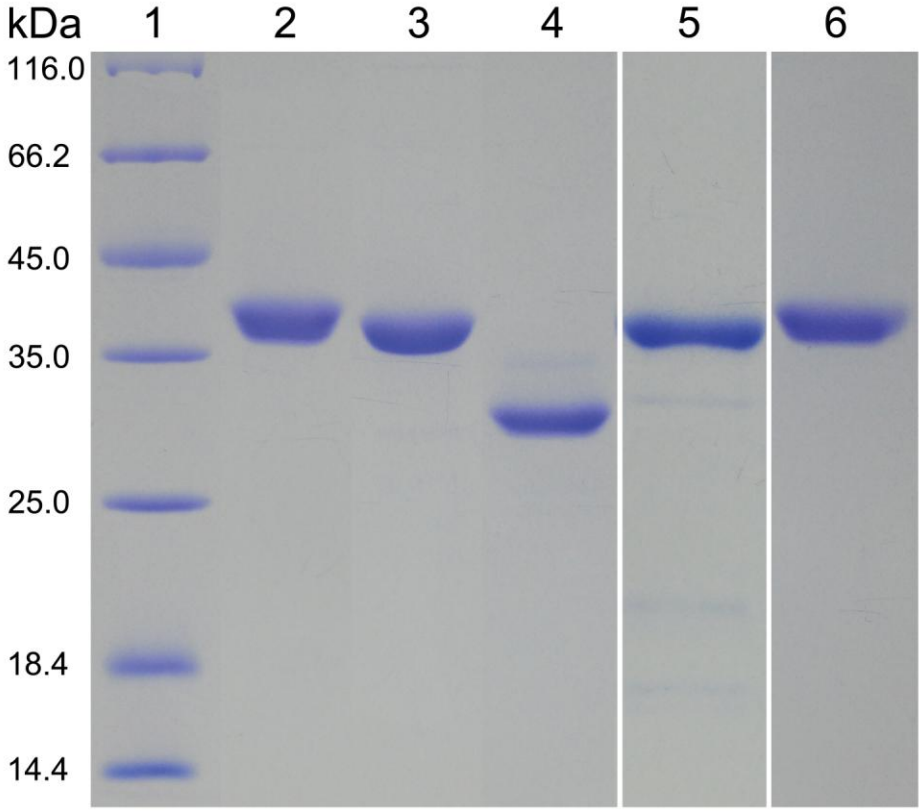


Figure S1 12% SDS-PAGE analysis of Nit2 proteins. Each purified protein was applied to individual lanes and stained with Coomassie Brilliant Blue. Lane 1, protein markers (Fermantas) containing *E. coli* β-galactosidase, bovine serum albumin, chicken ovalbumin, porcine lactate dehydrogenase, *E. coli* REase Bsp981, bovine β-lactoglobulin, chicken lysozyme. Lane 2, N-His_6-tagged yNit2. Lane 3, C-His_6-tagged yNit2. Lane 4, N-His_6-tagged hNit2. Lane 5, N-His_6-tagged R250Y mutant of yNit2. Lane 6, N-His_6-tagged C169S mutant of yNit2. This figure shows that the proteins used in the current work are of high purity (>99%). Approximate amounts of protein (μg) added to each lane are 15, 15, 4, 5, and 15 in lanes 2 through 6.

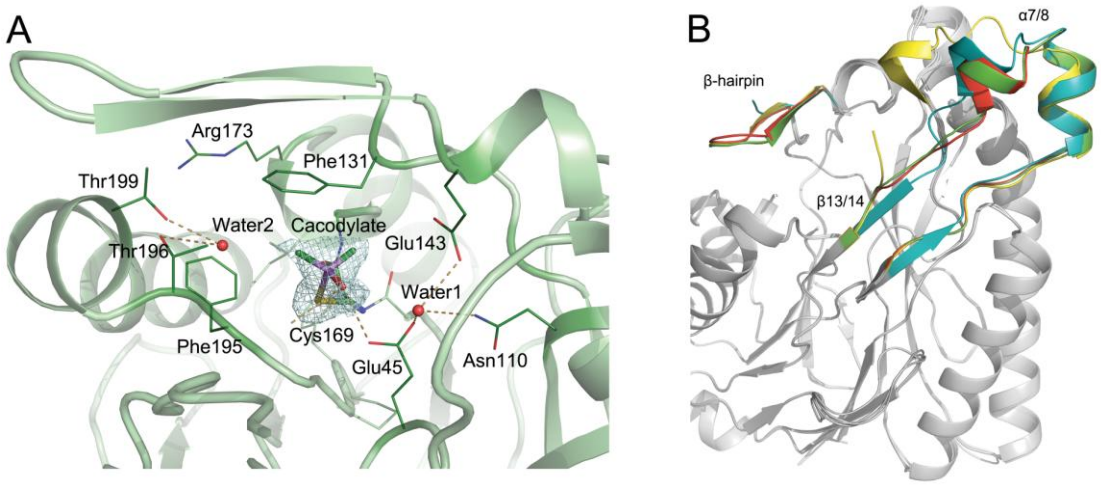


Figure S2 Structure of WT yNit2. A. Active site representation of WT yNit2. Crystallization of WT yNit2 in the presence of cacodylate buffer results in formation of a covalent adduct with the active site cysteine [$PSAs(=O)(CH_3)_2$]. The cystein-S-yl cacodylate in the active site, as was noted for α-KG, is covered in the deep cavity by the lid structure in the form of a β6/7-hairpin. Thr199 and Thr196 residues form hydrogen bonds with a water molecule, while the γ-carboxyl of Glu45 is hydrogen bonded to cystein-S-yl cacodylate directly. Glu45, Asn110 and Glu43 also form hydrogen bonds with a water molecule. These interactions are only partially similar to those noted for WT yNit2-α-KG. **B**. Structure of the monomer involved in the asymmetric unit of the yNit2 crystal. Different conformations are indicated by different colors. The main differences in the asymmetric unit are located at the β-hairpin and at α7/8, indicating their flexible properties.

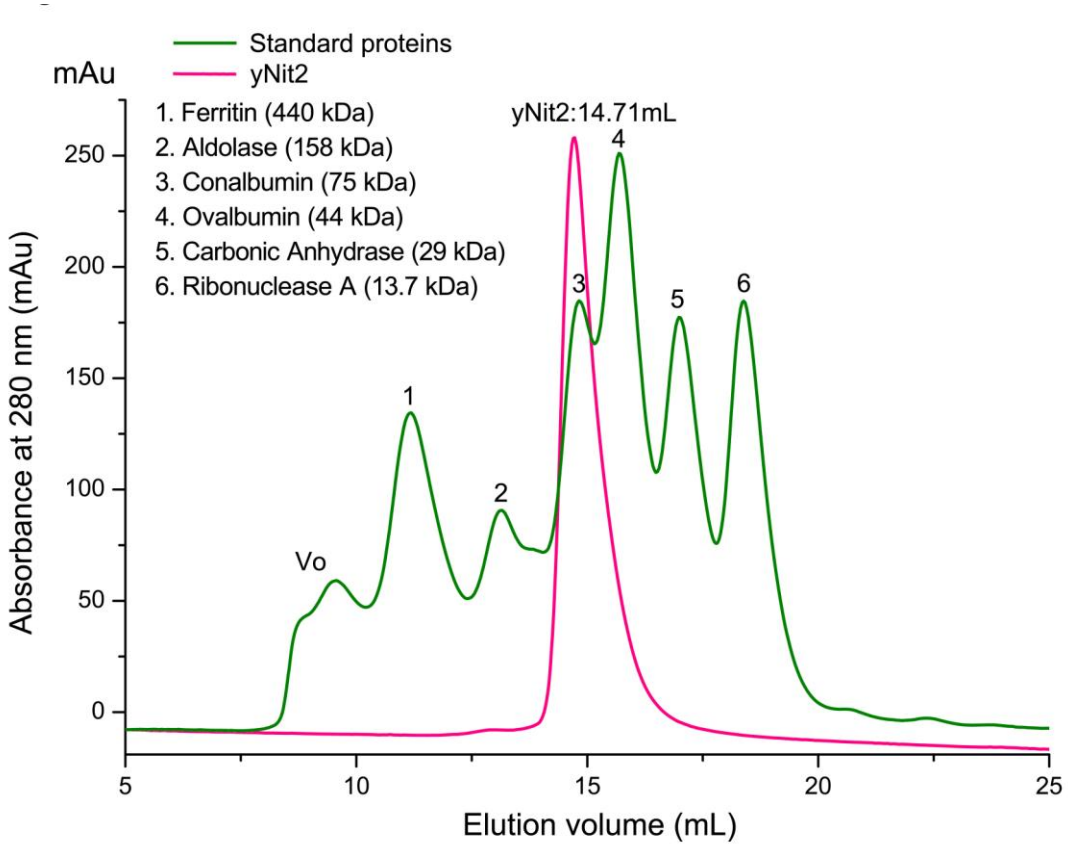
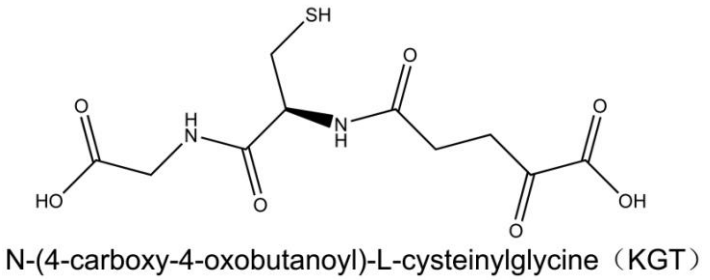


Figure S3 Chromatography of standard proteins and yNit2. The red trace represents the elution profile of purified yNit2 as determined by absorbance at 280 nm on a Superdex 200 16/60 GL column. The green trace shows the elution profile of standard proteins (GE Healthcare) with numbers above their peaks corresponding to those shown in the key. The peak of V0 (Blue Dextran 2000) is the void volume of the column.



N-(4-carboxy-4-oxobutanoyl)-L-cysteinylglycine（KGT）

Figure S4 Putative structure of the ligand bound to the mutant enzyme yNit2-C169S.

Table S1. Amino acid sequences of yNit2, recombinant yNit2, hNit2 and recombinant C169S and R250Y mutants.

WT yNit2 (UniProt ID: P47016)(307 amino acids) MTSKLKRVAVAQLCSSADLTKNLKVVKELISEAIQKKADVVFLPEASDYLSQNPLHSR YLAQKSPKFIRQLQSSITDLVRDNSRNIDVSIGVHLPPSEQDLLEGNDRVRNVLLYIDH EGKILQEYQKLHLFDVDVPNGPILKESKSVQPGKAIPDIIESPLGKLGSAICYDIRFPEFS LKLRSMGAEILCFPSAFTIKTGEAHWELLGRARAVDTQCYVLMPGQVGMHDLSDPE WEKQSHMSALEKSSRRESWGHSMVIDPWGKIIAHADPSTVGPQLILADLDRELLQEIR NKMPLWNQRRDDLFH
Recombinant WT N-His_6 tagged yNit2 (341 amino acids) MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSMTSKLKRVAVAQLCSSADLTK NLKVVKELISEAIQKKADVVFLPEASDYLSQNPLHSRYLAQKSPKFIRQLQSSITDLVR DNSRNIDVSIGVHLPPSEQDLLEGNDRVRNVLLYIDHEGKILQEYQKLHLFDVDVPNG PILKESKSVQPGKAIPDIIESPLGKLGSAICYDIRFPEFSLKLRSMGAEILCFPSAFTIKTG EAHWELLGRARAVDTQCYVLMPGQVGMHDLSDPEWEKQSHMSALEKSSRRESWG HSMVIDPWGKIIAHADPSTVGPQLILADLDRELLQEIRNKMPLWNQRRDDLFH MG…GS: cloning tag
Recombinant WT C- His_6 tagged yNit2 (315 amino acids) MTSKLKRVAVAQLCSSADLTKNLKVVKELISEAIQKKADVVFLPEASDYLSQNPLHSR YLAQKSPKFIRQLQSSITDLVRDNSRNIDVSIGVHLPPSEQDLLEGNDRVRNVLLYIDH EGKILQEYQKLHLFDVDVPNGPILKESKSVQPGKAIPDIIESPLGKLGSAICYDIRFPEFS LKLRSMGAEILCFPSAFTIKTGEAHWELLGRARAVDTQCYVLMPGQVGMHDLSDPE WEKQSHMSALEKSSRRESWGHSMVIDPWGKIIAHADPSTVGPQLILADLDRELLQEIR NKMPLWNQRRDDLFHLEHHHHHH LE…HH: cloning tag
Recombinant N-His_6 tagged yNit2 C169S mutant (341 amino acids) MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSMTSKLKRVAVAQLCSSADLTK NLKVVKELISEAIQKKADVVFLPEASDYLSQNPLHSRYLAQKSPKFIRQLQSSITDLVR DNSRNIDVSIGVHLPPSEQDLLEGNDRVRNVLLYIDHEGKILQEYQKLHLFDVDVPNG PILKESKSVQPGKAIPDIIESPLGKLGSAISYDIRFPEFSLKLRSMGAEILCFPSAFTIKTG EAHWELLGRARAVDTQCYVLMPGQVGMHDLSDPEWEKQSHMSALEKSSRRESWG HSMVIDPWGKIIAHADPSTVGPQLILADLDRELLQEIRNKMPLWNQRRDDLFH S: mutant site.
Recombinant N-His_6 tagged yNit2 R250Y mutant (341 amino acids) MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSMTSKLKRVAVAQLCSSADLTK NLKVVKELISEAIQKKADVVFLPEASDYLSQNPLHSRYLAQKSPKFIRQLQSSITDLVR DNSRNIDVSIGVHLPPSEQDLLEGNDRVRNVLLYIDHEGKILQEYQKLHLFDVDVPNG PILKESKSVQPGKAIPDIIESPLGKLGSAICYDIRFPEFSLKLRSMGAEILCFPSAFTIKTG EAHWELLGRARAVDTQCYVLMPGQVGMHDLSDPEWEKQSHMSALEKSSRYESWG HSMVIDPWGKIIAHADPSTVGPQLILADLDRELLQEIRNKMPLWNQRRDDLFH Y: mutant site.
Recombinant N-His_6 tagged hNit2 (286 amino acids)

MGSSHHHHHHMTSFRLALIQLQISSIKSDNVTRACSFIREAATQGAKIVSLPECFNSPY
GAKYFPEYAEKIPGESTQKLSEVAKECSIYLIGGSIPEEDAGKLYNTCAVFGPDGTLLA
KYRKIHLFDIDVPGKITFQESKTLSPGDSFSTFDTPYCRVGLGICYDMRFAELAQIYAQ
RGCQLLVYPGAFNLTTGPAHWELLQRSRAVDNQVYVATASPARDDKASYVAWGHST
VVNPWGEVLAKAGTEEAIVYSDIDLKKLAEIRQQIPVFRQKRSDLYAVEMKKP
MG…HH: cloning tag