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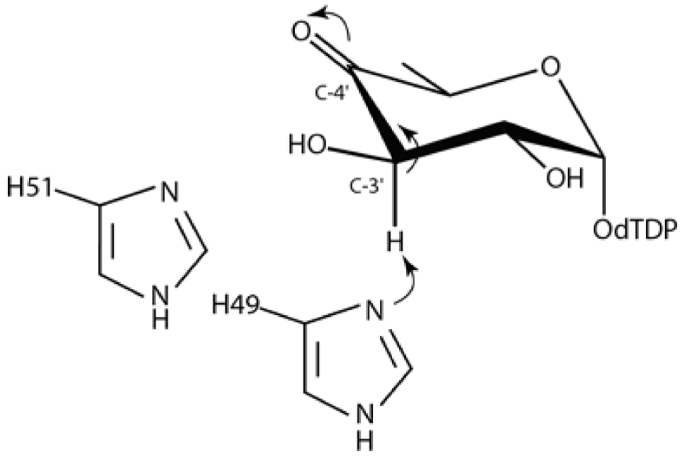
The questions on this exam explore Thoden, J.B., Vinogradov, E., Gilbert, M., Salinger, A.J., and Holden, H.M. (2015). Bacterial Sugar 3,4-Ketoisomerases: Structural Insight into Product Sterochemistry. *Biochemistry* **54:** 4495-4506.

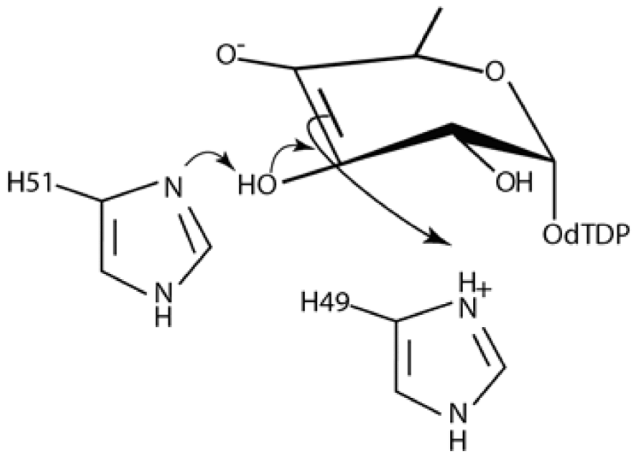
1. FdtA catalyzes the conversion of dTDP-4-keto-6-deoxyglucose to dTDP-3-keto-6-deoxygalactose. For each step of the conversion, **define the catalytic function of H49 and H51** where applicable as indicated by the image. Relevant modes of catalysis may include:

    - General acid catalysis
    - General base catalysis
    - Covalent catalysis
    - Metal ion catalysis
    - Electrostatic catalysis
    - Proximity and orientation effects
    - Preferential binding of the transition state

Step 1



Step 2



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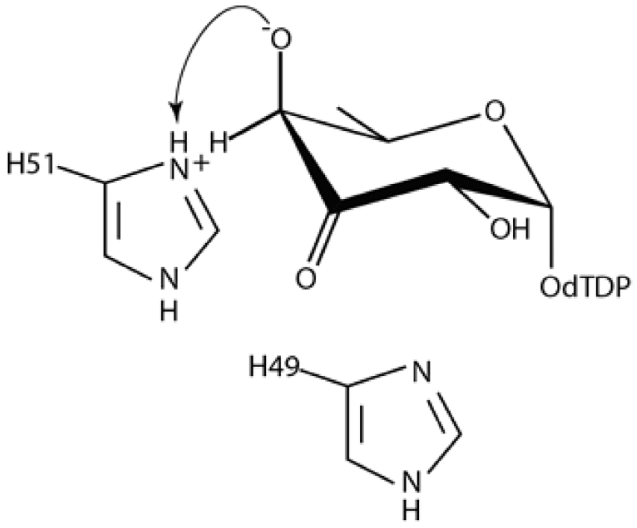
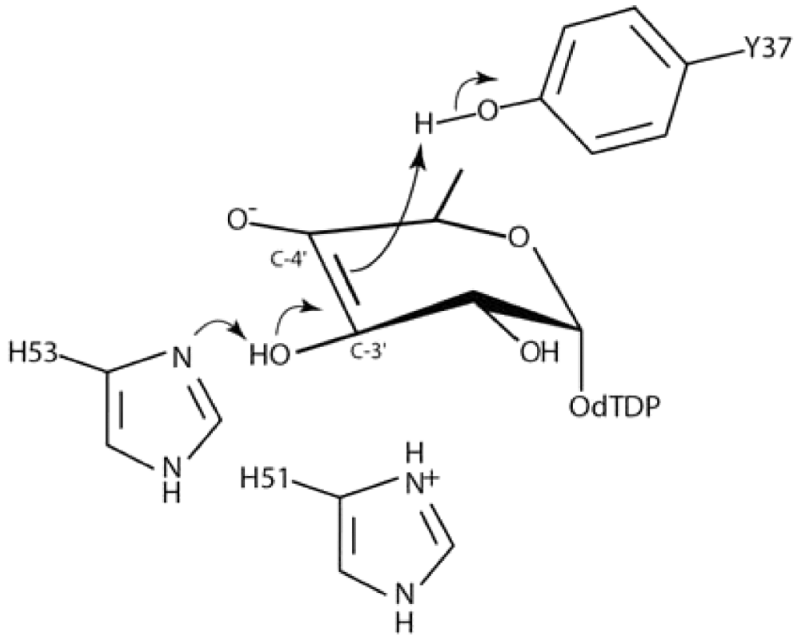
Step 3



2. QdtA catalyzes the conversion of dTDP-4-keto-6-deoxyglucose to dTDP-3-keto-6-deoxyglucose. An intermediate of the reaction mechanism for QdtA includes an oxyanion attached to C-4' (see image below). Draw and name the sidechain of an amino acid (other than H or Y) interacting with the oxyanion in the image below that could be used by the enzyme to stabilize this intermediate.



3. The authors understand that Y37 plays an important role in the catalytic mechanism of QdtA (see image in Question #2). The authors use site-directed mutagenesis to change Y37 to F37.

    a. Check all that apply to the sidechain of Y in general at pH 7.0, not just in the context of QdtA.

    ____ Hydrogen bond donor
    ____ Hydrogen bond acceptor
    ____ Proton donor
    ____ Proton acceptor
    ____ Aromatic
    ____ Nonpolar
    ____ Polar
    ____ Nucleophile
    ____ Electrophile

    b. Check all that apply to the sidechain of F in general at pH 7.0, not just in the context of the mutant QdtA.

    ____ Hydrogen bond donor
    ____ Hydrogen bond acceptor
    ____ Proton donor
    ____ Proton acceptor
    ____ Aromatic
    ____ Nonpolar
    ____ Polar
    ____ Nucleophile
    ____ Electrophile

    c. Do you consider the mutation of Y37 to F to be conservative (e.g., moderate and cautious) or extreme? Explain your reasoning.

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4. The authors observed the following kinetic data for wildtype QdtA.

   a. Use Excel to determine the V_{max} and K_M parameters for the data. Round your final values appropriately.

Initial [S] (uM)	Initial Rate (uM/min)
25	10
50	18
100	26
200	32
500	47
750	50
1000	53
1500	56
2000	58

V_{max} = _____(±_____) μM min^{-1}

K_M = _____(±_____) μM

   b. The authors report the k_{cat} to be 231 min^{-1}. Determine the total enzyme concentration.

$[E]_{total}$ = _____ μM

   c. Imagine that a competitive inhibitor of QdtA was found and studied. If the equilibrium dissociation constant (K_I) for the inhibitor is 1000 μM, fill in the following table.

Initial [S] (uM)	Initial Rate (uM/min)	[I] (uM)
25	10	0
50	18	0
100	26	0
200	32	0
500	47	0
750	50	0
1000	53	0
1500	56	0
2000	58	0
25		4000
50		4000
100		4000
200		4000
500		4000
750		4000
1000		4000
1500		4000
2000		4000

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For this portion of the final exam, you will be determining the specific activity of a β-galactosidase enzyme preparation. To begin, make sure that you have:

- One microfuge tube with 11.61 mM ONGP in assay buffer (red dot)
- One microfuge tube with 0.326 mg ml^{-1} β-galactosidase (blue dot)
- One conical tube with assay buffer
- One 1 mL cuvette

1. Prepare for a kinetic assay by calculating the amount of each component that will be required to produce exactly 1 mL total volume with (do not round):

    - 3.0 mM ONGP _____ **µL 11.61 mM ONGP**
    - 3.26 µg β-galactosidase _____ **µL 0.326 mg ml^{-1} β-galactosidase**

    _____ **µL assay buffer**

Perform the kinetic assay suggested in question #1 monitoring the rate of absorbance increase at 410 nm.

2. Draw a sketch of the result from LoggerPro for your assay. Include axis labels and values.

3. Use Excel to determine the rate by which absorbance increased in both time units:

    _____ **s^{-1}**

    _____ **min^{-1}**

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4. Determine the rate of ONP production. The extinction coefficient of ONP is 4.8 mM^{-1} cm^{-1}.

_____ mM min^{-1}

5. Determine the activity within the assay.

_____ mU mU = nmole min^{-1}

6. Determine the specific activity of the enzyme preparation.

_____ mU per mg of enzyme

- Close LoggerPro without saving.
- Close Excel without saving.
- Pour your solutions down the drain with water.
- Dispose of the tube.

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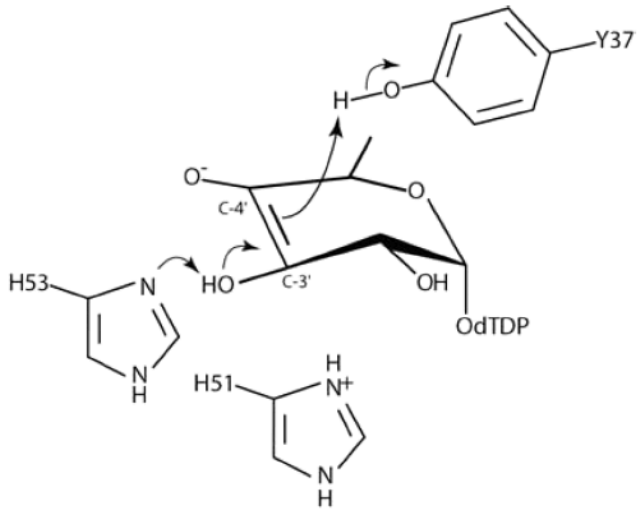
1. Open the structure of QdtA Y17R/R97H (PDB ID: 4ZU7) in PyMol.

The authors suggest that H51; H53; and Y37 are important in the catalytic mechanism of QdtA.



2. Determine the shortest distance between:
    a. Amine nitrogen atoms of H51 and H53.

    b. An amine nitrogen atom of H51 and an oxygen atom of Y37.

    c. An amine nitrogen atom of H53 and an oxygen atom of Y37.

3. Y38 is not suggested to play a catalytic role in QdtA, while the catalytic rate constant is decreased by 167-fold when Y37 is mutated. Explain why the adjacent and chemically identical amino acid, Y38, is not catalytically important.