

SUPPORTING INFORMATION

Isopentenyl Diphosphate Isomerase Catalyzed Reactions in D₂O: Product Release Limits the Rate of this Sluggish Enzyme-Catalyzed Reaction

Venkatadurga Jonnalagadda, Krisztina Toth, and John P. Richard*

[†]Department of Chemistry, University at Buffalo, SUNY, Buffalo, NY 14260, USA

EXPERIMENTAL

Materials. Isopentenyl pyrophosphate was prepared by a published procedure.¹ The following deuterium labeled solvents and reagents were purchased from Cambridge Isotope Laboratories: D₂O, CDCl₃, DCl (35% wt, 99.9% D), and NaOD (30% wt, 99.9% D). All other organic and inorganic chemicals were reagent grade and were used without further purification.

NMR analysis. All ¹H NMR spectra were acquired on a Varian Unity Inova-500 spectrometer that had been shimmed to give a line width of ≤ 0.8 Hz for the most downfield peak of the double triplet for the C-1 proton of [1-¹³C]-glycolaldehyde.² Chemical shifts were reported relative to the reference HOD peak at 4.67 ppm. Spectra (4 – 32 transients) were obtained using a sweep width of 6000 Hz, a pulse angle of 90°, an acquisition time of 6 s and a relaxation delay between pulses of 60 s, which is > 9T₁ for the protons of IPP and DMAPP. The concentration of the hydrogen atoms present in the reactant IPP and product DMAPP was calculated from the relative area for the peak(s) of interest and the area of the singlet for 1 mM tetramethyl ammonium sulfate that was present as an internal standard. The concentrations of DMAPP and IPP calculated from these integrated peak areas show that there is good mass balance during IDI-catalyzed isomerization of IPP to DMAPP. Inverse gated homonuclear decoupling was carried

out as described in an earlier study of the deuterium exchange reaction of the $-\text{CH}_2-$ group of $\text{CH}_3\text{CH}_2\text{CN}$,³ by irradiating the C-1 protons at 3.96 during the spectral acquisition.

Preparation of Solutions. The pH and pD were measured at 25 °C on an Orion Model 720A pH meter equipped with Radiometer pHC4006-9 combination electrode standardized at pH 7 and 10. The pD of the buffers prepared in deuterium oxide was obtained by adding 0.4 to the reading on the pH meter.⁴ Buffered solutions were prepared by dissolving the corresponding base in D_2O and adjusting to the required the pD using DCl.

Enzyme Assay. Isopentenyl diphosphate isomerase (IDI) was assayed by monitoring the appearance of the signal for the Z-methyl protons of DMAPP by ^1H NMR and using the C(4,5) hydrogen of imidazole as an internal standard. The enzyme concentration was determined from the absorbance at 280 nm and using a protein extinction coefficient of $\epsilon_{280} = 40300 \text{ M}^{-1} \text{ cm}^{-1}$.⁵ A standard pulse sequence was used to suppress the signal for the water protons. The assay contained 20 mM IPP at pH 7.4 (20 mM imidazole), 0.20 M KCl, 10 mM MgCl_2 , 0.5 mM DTT in a volume of 0.70 mL at 25 °C. The reaction was initiated by the addition of 10 μL of a stock solution of IDI to give a final enzyme concentration of 3.5 μM . ^1H NMR spectra were obtained every 5 minutes for a period of 1 h during which time *ca* 5% of the substrate was converted to product. The initial reaction velocity, determined as the slope of a plot of product concentration against time, was used to calculate a value of $k_{\text{cat}} = 0.08 \text{ s}^{-1}$. This is similar to $k_{\text{cat}} = 0.13 \text{ s}^{-1}$ calculated from specific activity of IDI-catalyzed reaction of IPP in H_2O at 37 °C.⁵

IDI-Catalyzed Isomerization of IPP in D_2O . Two IDI-catalyzed reactions of IPP in D_2O were carried out at 25 °C and pD 7.4. The IDI was prepared as stock solutions at pD 7.4 (20 mM imidazole) that contained 400 mM NaCl. (A) The reaction was initiated by mixing 350 μL of a stock solution of 56 μM IDI and 350 μL of a buffered solution of substrate and other

necessary reagents to give a final solution that contained 28 μM IDI, 26.5 mM IPP, 10 mM MgCl_2 0.5 mM dithiothreitol, 1 mM tetramethyl ammonium sulfate and 400 mM NaCl at pD 7.4 (20 mM imidazole buffer). (B) The reaction was initiated by mixing 500 μL of a stock solution of 100 μM IDI and 200 μL of a solution of a buffered solution of substrate and other necessary reagents to a final solution of 71 μM IDI, 9 mM IPP, 10 mM MgCl_2 0.5 mM dithiothreitol, 1 mM tetramethyl ammonium sulfate and 400 mM NaCl at pD 7.4 (20 mM imidazole buffer). Reaction A was monitored periodically by ^1H NMR for a period of 10 h, and reaction B was monitored for 18 h. In each case, the protein was removed by ultrafiltration at the end of the reaction and the pD of the solution was determined. There was no significant change in pD during either reaction.

^1H NMR spectra were obtained with inverse gated homonuclear decoupling of the C-1 and C-2 protons by irradiating the C-1 protons at 3.96 during the spectral acquisition. The signals for the following protons were monitored. IPP: CH_3 , 1.69 ppm; 2- CH_2 -, 2.295 ppm; 2-CHD-, 2.272 ppm. It was not possible to cleanly resolve the vinylic protons of IPP from the signal for HOD at 4.67 ppm. DMAPP: Z- CH_3 , 1.66 ppm, E- CH_2D , 1.63 ppm; 1- CH_2 -, 4.35 ppm; 2-CH=, 5.34 ppm.

REFERENCES

- (1) Davisson, V.; Woodside, A.; Neal, T.; Stremmer, K.; Muehlbacher, M.; Poulter, C. *J. Org. Chem.* **1986**, *51*, 4768-4779.
- (2) Go, M. K.; Amyes, T. L.; Richard, J. P. *Biochemistry* **2009**, *48*, 5769-5778.
- (3) Richard, J. P.; Williams, G.; Gao, J. L. *J. Am. Chem. Soc.* **1999**, *121*, 715-726.
- (4) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188-90.
- (5) Carrigan, C. N.; Poulter, C. D. *J. Am. Chem. Soc.* **2003**, *125*, 9008-9009.