Characterization of Urea Hydrolysis in Fresh Human Urine and Inhibition by Chemical Addition

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SUPPLEMENTARY MATERIALS

12 pages
4 tables
9 figures
1. Material and methods

1.1. Batch chemical addition experiments

Fourteen separate beakers each containing a magnetic stir bar were filled with 75 mL of fresh urine and placed on the multistir plate in four rows of three and one row of two. The pH and conductivity of the 14 samples were measured before the start of the experiment for $t = 0$ min reading. A timer was set for 15 min and the stirplate was set to 350 rpm speed. At the start of the timer, 2.5 mL of the highest concentration of inhibitor was added to each beaker in the first row, next 2.5 mL of the middle concentration of inhibitor was added to each beaker in the second row, and 2.5 mL of the lowest concentration of inhibitor was added to each beaker in the third row. The fourth row contained only urine and inhibitor and thus the highest concentration inhibitor was added to the first beaker, the middle concentration inhibitor was added to the second beaker, and the lowest concentration inhibitor was added to the third beaker in the row. This row did not receive any urease. The fifth row of two beakers did not receive any inhibitor as one beaker serves as a urine only control and the other beaker served as a urine and urease control. At $t = 15$ min, the pH and conductivity of each sample was recorded and the pre-weighed urease batches were added to the first three rows and to the first sample in the fifth row. The first three rows containing urease were the samples simulating hydrolysis and the inhibition of the inhibitor was being observed for the three different concentrations. The fourth row did not contain any urease and were urine and inhibitor only controls. The first sample in the fifth row containing urine and urease only served as a positive control to track uninhibited hydrolysis. The second sample in the fifth row was urine only, which served as a negative control. The pH and conductivity of each sample was recorded every 15 min for 240 min and each inhibitor dose was tested in triplicate as described above.
1.2. Analytical methods

For the ammonia monitoring during urea hydrolysis and urea monitoring during hydrolysis experiments, 1 L of synthetic fresh urine was prepared. For the pH monitoring during urea hydrolysis, conductivity monitoring during urea hydrolysis, and chemical addition inhibition experiments, 2 L of synthetic urine was prepared. For each experiment, about 1400 mL of fresh urine was obtained.

The ammonia probe was calibrated to six calibration points, 5, 425, 825, 1700, 5000, and 8700 mg/L as NH₃, following the detailed instructions found in the probe manual. The 5, 425, 825, and 1700 mg/L calibration standards were prepared using the 0.1 M ammonium chloride standard (CAS 13-641-923, Fisher Scientific) and the 5000 and 8700 mg/L calibration standards were prepared using ammonium chloride (CAS A649-500, Fisher Scientific). 0.0346 (±0.0002) g of sodium chloride (CAS S641-500, Fisher Scientific) was added to the 5, 425, 825, and 1700 mg/L calibration standards. 0.8646 (±0.0002) g of sodium chloride was added to the 5000 mg/L calibration standard and 0.8638 (±0.0002) g was added to 8700 mg/L calibration standard. The sodium chloride was added to the calibrations standards following the instructions in the ammonia probe manual. The calibration standards should have an ionic strength similar to solution being measured from, which in this case was urine. After the probe was calibrated, the accuracy was checked by measuring a 10 mg/L sample made from the 1000 mg/L ammonia as N standard (CAS 13-641-924C). The ammonia concentration was measured following the detailed instructions in the ammonia probe manual.

1.3. Data analysis

Visual MINTEQ 3.1 was used for the ionic zinc, ionic silver, and fluoride addition experiments to determine if precipitation would occur at the added concentrations. For each
inhibitor, the individual concentrations of individual species detailed in Table S2 were entered into the software. For the ionic zinc and ionic silver, the component concentrations were altered to match the altered synthetic urine used in certain experiments. The chemical inhibitor concentrations were then entered into the software. The software was run at both a fixed pH of 6 as well as with the pH being calculated from the mass balance. The software produced saturation indices for different minerals where a negative number represents an undersaturation and a positive number represents an oversaturation of the mineral.

2. Results

Table S1 – Synthetic fresh urine composition

| Compound   | Concentration (g/L) |
|------------|---------------------|
| Urea       | 15.0075             |
| NaCl       | 2.5715              |
| Na₂SO₄     | 2.1305              |
| KCl        | 2.982               |
| MgCl₂·6H₂O | 0.813               |
| NaH₂PO₄    | 2.3995              |
| CaCl₂·2H₂O | 0.588               |
| pH         | 6                   |

Table S2 – Concentrations of individual species in synthetic fresh urine

| Compound | Concentration (mmol/L) |
|----------|------------------------|
| N        | 500                    |
| Na       | 94                     |
| Cl       | 100                    |
| K        | 40                     |
| PO₄      | 20                     |
| Ca       | 4                      |
| Mg       | 4                      |
| SO₄      | 15                     |
### Table S3 – Visual MINTEQ 3.1 saturation indices

| Inhibitor\(^a\) | Concentration of inhibitor | Mineral | Saturation index |
|------------------|---------------------------|---------|-----------------|
| **Zinc Nitrate (meq/L as Zn\(^{2+}\))** | 2.16E-01 | Zn\(_3\)(PO\(_4\))\(_2\):4H\(_2\)O | 2.20 |
| | 2.16 | Zn\(_3\)(PO\(_4\))\(_2\):4H\(_2\)O | 5.19 |
| | 2.02E+01 | Zn\(_3\)(PO\(_4\))\(_2\):4H\(_2\)O | 8.14 |
| **Silver Nitrate (meq/L as Ag\(^{+}\))** | 3.23E-04 | AgCl | -1.08 |
| | 3.23E-03 | AgCl | -0.076 |
| | 3.23E-02 | AgCl | 0.924 |
| **Sodium Fluoride (meq/L as F\(-\))** | 3.23E-02 | CaF\(_2\) | -1.91 |
| | 3.23E-01 | CaF\(_2\) | 0.089 |
| | 3.23 | CaF\(_2\) | 2.09 |

\(^a\) All concentrations represent the concentration of the inhibitor in the beaker

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### Table S4 – Concentrations of six common metabolites in human urine

| Compound \(^a\) | Concentration (mmol/L) |
|------------------|------------------------|
| Creatinine \(^a\) | 12.246 |
| Glycine \(^a\) | 1.2368 |
| Taurine \(^a\) | 0.9919 |
| Hippurate \(^a\) | 2.8043 |
| Citrate \(^a\) | 2.486 |
| L-Cysteine \(^a\) | 0.8058 |

\(^a\) Bouatra et al. (2013)
Fig. S1. Urea hydrolysis simulation using four doses of Jack Bean urease. The legend indicates the concentration of Jack Bean urease utilized. The experiments were performed using 75 mL of synthetic, fresh urine. Data are mean ± one standard deviation for triplicate samples.
Fig. S2. Urea hydrolysis inhibition utilizing 0.533 g/L of Jack bean urease and zinc nitrate and silver nitrate as the inhibitor. Legend indicates the concentration of added chemical inhibitor in urine the different synthetic, fresh urine types. All experiments were performed using 75 mL of synthetic, fresh urine. The experiments start with urine and the chemical inhibitor mixing at time -15 min. Time 0 min represents the time when urease is added. (a) Represents the pH vs time for the zinc nitrate in synthetic, fresh urine containing phosphate and (b) represents the pH vs. time for zinc nitrate in synthetic, fresh urine with phosphate replaced by NaCl. (c) Represents the pH vs time for the silver nitrate in synthetic, fresh urine containing chloride and (d) represents the pH vs. time for silver nitrate in synthetic, fresh urine with all chloride compounds replaced with corresponding nitrate compounds. For the zinc nitrate experiment, the additional sodium chloride concentration was determined by adding the corresponding chloride molar concentration of phosphate that was removed. For the silver nitrate experiment, all chloride compounds were replaced with corresponding nitrate compounds (i.e., sodium nitrate for sodium chloride). Data are mean ± one standard deviation for triplicate samples. Corresponding conductivity plots in Fig. 3.
Fig. S3. Urea hydrolysis inhibition utilizing 0.533g/L of Jack bean urease and sodium fluoride as the inhibitor. Legend indicates the concentration of added chemical inhibitor in either synthetic or real, fresh urine. All experiments were performed using 75 mL of urine either synthetic or real, fresh urine. The experiments start with urine and the chemical inhibitor mixing at time -15 min. Time 0 min represents the time when urease is added. (a) shows the trend of pH vs time in synthetic, fresh urine and (b) shows the trend of pH vs time in real, fresh urine. Data are mean ± one standard deviation for triplicate samples. Corresponding conductivity plots in Fig. 4.
Fig. S4. Urea hydrolysis inhibition utilizing 0.533 g/L of Jack bean urease and sodium fluoride as the inhibitor. Legend indicates the concentration of added chemical inhibitor in synthetic, fresh urine with all calcium removed and replaced with the equivalent chloride molar concentration in the form of sodium chloride. All experiments were performed using 75 mL of the altered synthetic, fresh urine. Experiments start with urine and the chemical inhibitor mixing at time equal to $-15$ min. Time equal to 0 min represents the time when urease is added. (a) Conductivity vs. time in altered synthetic, fresh urine and (b) pH vs. time in altered synthetic, fresh urine. Data are mean ± one standard deviation for triplicate samples.

Fig. S5. Urea hydrolysis inhibition utilizing 0.533 g/L of Jack bean urease and sodium fluoride as the inhibitor. Legend indicates the concentration of added chemical inhibitor in synthetic, fresh urine with metabolites added. Refer to Table S3 for the metabolite compound and concentrations. All experiments were performed using 75 mL of synthetic, fresh urine. Experiments start with urine and the chemical inhibitor mixing at time equal to $-15$ min. Time equal to 0 min represents the time when urease is added. (a) Conductivity vs. time in synthetic, fresh urine and (b) pH vs. time in real, fresh urine. Data are mean ± one standard deviation for triplicate samples.
Fig. S6. Urea hydrolysis inhibition utilizing 0.533g/L of Jack bean urease and acetic acid and sulfuric acid as the inhibitor where the inhibitor is added before the urease. All experiments were performed using 75mL of synthetic, fresh urine. Legend indicates the concentration of added chemical inhibitor in synthetic, fresh urine. The experiments start with urine and the chemical inhibitor mixing at time -15 min. Time 0 min represents the time when urease is added. (a) and (b) represent acetic acid in synthetic, fresh urine, (c) and (d) represent sulfuric acid, (e) and (f) also represent sulfuric acid but at a different concentration range. Data are mean ± one standard deviation for triplicate samples.
Fig. S7. Urea hydrolysis inhibition utilizing 0.533 g/L of Jack bean urease and acetic acid and sulfuric acid as the inhibitor where the inhibitor is added after the urease. Legend indicates the concentration of added chemical inhibitor in synthetic, fresh urine. All experiments were performed using 75 mL of synthetic, fresh urine. The experiments start with urine and urease mixing at time 0 min. Time 15 min represents the time when the acid is added. (a) Represents the conductivity vs. time for the acetic acid in synthetic, fresh urine and (b) represents the pH vs. time for acetic acid in synthetic, fresh urine. (c) Represents the conductivity vs time for sulfuric acid in synthetic, fresh urine and (d) represents the pH vs. time for sulfuric acid in synthetic, fresh urine. Data are mean ± one standard deviation for triplicate samples.
Fig. S8. Urea hydrolysis inhibition utilizing 0.533g/L of Jack bean urease and acid as the inhibitor. Legend indicates the concentration of added chemical inhibitor in either synthetic or real, fresh urine. All experiments were performed using 75mL of either synthetic or real, fresh urine. The experiments start with urine and the chemical inhibitor mixing at time -15 min. Time 0 min represents the time when urease is added. Open symbols represent experiments using synthetic fresh urine and solid symbols represent experiments using real fresh urine. Each row represents a different chemical acid inhibitor. (a) and (b) acetic acid, (c) and (d) 6% cleaning vinegar, (e) and (f) citric acid. Data are mean ± one standard deviation for triplicate samples. Corresponding conductivity plots in Fig 5.
Fig. S9. Urea hydrolysis inhibition utilizing 0.533g/L of Jack bean urease and vinegar as the inhibitor. Legend indicates the concentration of different vinegars in synthetic, fresh urine. All experiments were performed using 75 mL of synthetic, fresh urine. The experiments start with urine and the chemical inhibitor mixing at time -15 min. Time 0 min represents the time when urease is added. (a) shows the trend of conductivity vs time in synthetic, fresh urine and (b) shows the trend of pH vs time in synthetic, fresh urine. Data are mean ± one standard deviation for triplicate samples.