

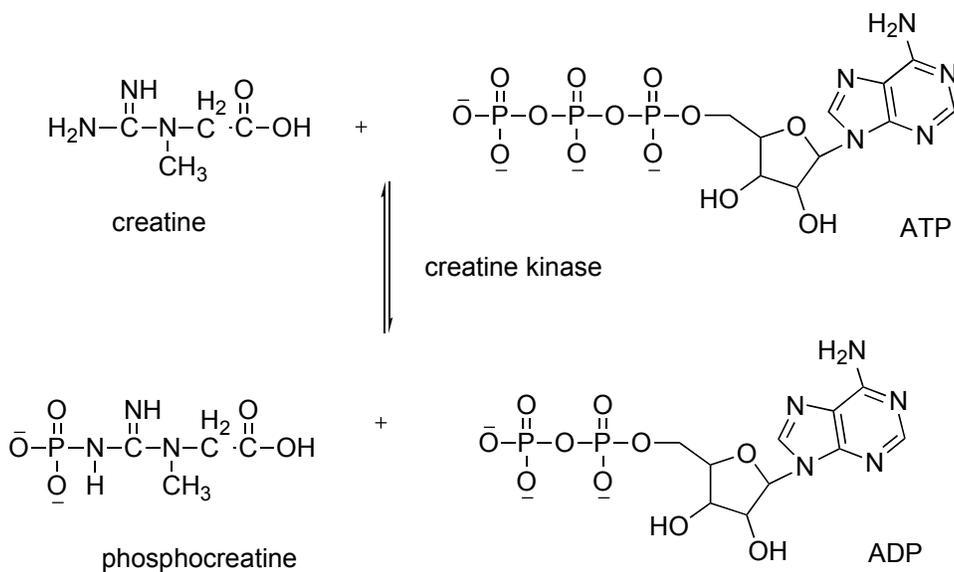
## Synthesis of Creatine

Adapted by Timothy Nowak (PSU '07) from a procedure given by Andri L. Smith and Paula Tan (J. Chem. Ed. 2006, 83, 1654-1655)

### Introduction:

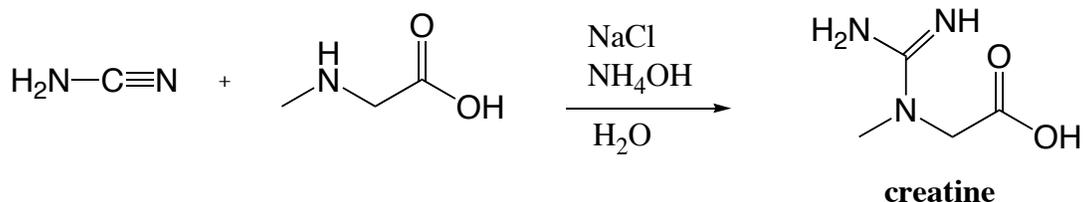
Creatine is a naturally occurring organic acid that is produced in body by the liver, kidneys, and pancreas. Creatine can be absorbed from food, particularly from meat and fish. When food intake is low, creatine is produced from the amino acids: glycine, arginine, and methionine in the liver, kidneys, and pancreas. Michel Eugène Chevreul discovered creatine from skeletal muscles and named it creatine after the Greek word *Kreas* meaning flesh.<sup>1</sup> Creatine provides the energy necessary for vigorous muscle contraction and has been shown to enhance performance in high-intensity exercise. Creatine supports high energy metabolism of ATP for a short periods of time.<sup>2</sup>

Creatine exists in equilibrium with phosphocreatine, the reversible conversion of creatine into phosphocreatine is catalyzed by the enzyme creatine kinase.<sup>1</sup> Creatine phosphate is an ATP-synthesizer which serves as a reservoir of high-potential phosphoryl groups that can be readily transferred to ATP. During strenuous exercise, creatine phosphate regenerates ATP from ADP from creatine kinase.<sup>3</sup> The reaction is expressed below.



Creatine increases anaerobic capacity, aerobic recovery, and protein synthesis. In animal studies, it has been shown to give positive results on treatment of traumatic brain injury and Lou Gehrig's disease. Also, LaVonne Veatch Goodman, M.D. reported in January 2006 that creatine has positive effects on Huntington's Disease. It has been reported that creatine is bioavailable in brain and reduces serum.<sup>4</sup>

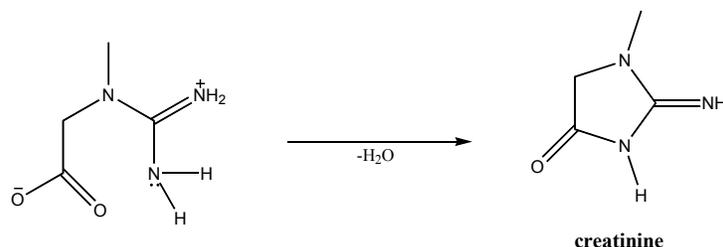
After scientists discovered creatine is stored in intramuscular and plays a role in muscular metabolism, the drug went commercial. Creatine has been used in dietary supplements and has been implemented as supplements to athletes. Although it is not regulated by the FDA, it is not banned by National Collegiate Athletic Association or the International Olympic Committee.<sup>2</sup> It is widely used among several age group athletes. A University of Wisconsin Sports Medicine researched in a survey given to high school student athletes. About 25.3% of students reported using creatine as a supplement. More upper level high school students reported using creatine as a supplement compared to freshman (50.5% : 10.4%).<sup>2</sup> The synthesis of creatine is shown in Scheme 1.



Scheme 1. Synthesis of creatine

Prelaboratory Exercise:

1. What does ATP stand for? In a biological chemistry perspective, what is the purpose of ATP?
2. Creatinine is created by creatine. Creatine is converted by an irreplaceable process. Draw a mechanism (shown in Figure 3) to show the transformation of creatine to creatinine.



*Caution!* Sarcosine is hygroscopic, while cyanamide is highly toxic and corrosive. Concentrated ammonium hydroxide causes severe skin irritation, severe irritation to the respiratory tract, and is toxic. Gloves and safety goggles should be worn at all times when dealing with the chemicals. All work should be done in the hood.

Synthesis:

To a 10 mL round-bottom flask, add 232 mg of sarcosine and 0.5 mL distilled water. Then, add 152 mg of NaCl to the flask. Add a microscale stir bar and stir the mixture. In a small beaker or shorty vial, add 206 mg of cyanamide and 0.2 mL of distilled water. Then, add a drop of concentrated ammonium hydroxide. Swirl the container, and then add the cyanamide mixture to the sarcosine mixture. Stir the new mixture for one hour. Let the mixture sit for one week. After one week, the product should precipitate. If it is still in solution, vacuum filter the solution until the crystals are dry. Then, add the crystals to a clean 10mL Erlenmeyer Flask. If some crystals came out of solution, filter again and add the crystals to the flask.

Isolation and Purification:

Recrystallize the white crystals using 1-2 mL of boiling distilled water. Then cool the solution until it reaches room temperature. Then, cool it in an ice bath for five minutes. Isolate the precipitated by vacuum filtering via Hirsch Funnel washing with small portions of distilled water. Transfer the white needle-like crystals to a tared watch glass, allow to dry, and weigh determining the percent yield.

Clean-up:

All aqueous solutions can be washed down the drain with sufficient amounts of cold running water. After analysis are complete, dispose of the synthesized creatine in a waste basket.

#### Analysis:

Determine the purity by taking a melting point of creatine and comparing it to the literature values. Purity can also be determined by TLC analysis using a 3:1 *n*-propanol:water solvent system. A TLC stain, potassium permanganate) may be needed to visualize the spots. Dip the plate in the solution of KMnO<sub>4</sub>, blot the plate on a paper towel to absorb excess stain, then hold over a hot plate using forceps. Spots should be visible. Finally, obtain a <sup>1</sup>H-NMR using D<sub>2</sub>O as a solvent (can be found from the stockroom) and obtain an IR spectrum.

#### Final report:

Discuss any observed observations seen during the experiment. Comment on purity and yield of the final product.

#### Post lab questions:

1. Creatine would still form if NaCl and Ammonium hydroxide are removed. Comment on what these two chemicals are used for in this experiment.
2. How would you improve on this experiment?

#### References:

1. <http://en.wikipedia.org/wiki/Creatine>
2. Smith A.L; Tan P.; *J. Chem. Ed.* **2006**, 83, 1654-1655
3. Berg, J.M.; Tymoczko J. L.; Stryer L.; *Biochemistry*, 6<sup>th</sup> edition, W.H. Freeman and Company: New York, 2007
4. [http://www.hdac.org/features/article.php?p\\_articleNumber=219](http://www.hdac.org/features/article.php?p_articleNumber=219)