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Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

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NAC: It's Not Just for Glutathione Anymore

by

Sherry A. Rovinsky

5-607 Bowen Science Building
Department of Physiology and Biophysics
The University of Iowa
Iowa City, IA 52242-1109

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Abbreviations

GSH, glutathione
GSSG, glutathione disulfide
$HO^{\bullet}$, hydroxyl radical
H_2O_2, hydrogen peroxide
HOCl, hypochlorous acid
HPLC, high-performance liquid chromatography
HRP, horseradish peroxidase
NAC, N-acetyl-L-cysteine
NPM, N-(1-pyrenyl)maleimide
$O_2^{\bullet-}$, superoxide anion
RSH, N-acetylcysteine
RSSR, N-acetylcysteine disulfide

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Abstract

N-acetyl-L-cysteine (NAC) is a thiol antioxidant that has many therapeutic and experimental uses. It is a glutathione precursor, but is also a powerful antioxidant in its own right. NAC is an excellent scavenger of hypochlorous acid and hydroxyl radical. It also reduces hydrogen peroxide and superoxide, although much more slowly. Recently, reversed-phase high-performance liquid chromatography methods have been developed to quantify NAC in biological material.

Introduction

N-acetyl-L-cysteine is a small, water-soluble [1], thiol-containing antioxidant that has been used both experimentally and clinically since the 1950s [2]. It was originally used as a mucolytic agent in congestive and obstructive lung diseases such as cystic fibrosis and chronic bronchitis, diseases characterized by hypersecretion of respiratory mucus [2]. More recently, it has been used for treatment of acetaminophen overdose [3]. Experimentally, it has been used as an antioxidant, in particular to decrease damage to cell components by oxidants [2, 4]. NAC primarily acts to scavenge hydroxyl radical ($HO^{\bullet}$) and hypochlorous acid (HOCl), but also reacts slowly with hydrogen peroxide (H_2O_2) [5] and superoxide ($O_2^{\bullet -}$) [6]. N-acetylcysteine also enhances glutathione (GSH) pools. It is a GSH precursor and also increases the efficacy of such enzymes as GSSG reductase. This enzyme recycles glutathione disulfide (GSSG) back to GSH [7].

Background

N-acetylcysteine is a small (MW = 163.20), stable molecule with a melting point of 109-110°C [3]. It is a derivative of the amino acid cysteine with an acetyl group linked to the nitrogen. One of the ways NAC can contribute to increased GSH levels is through deacetylation

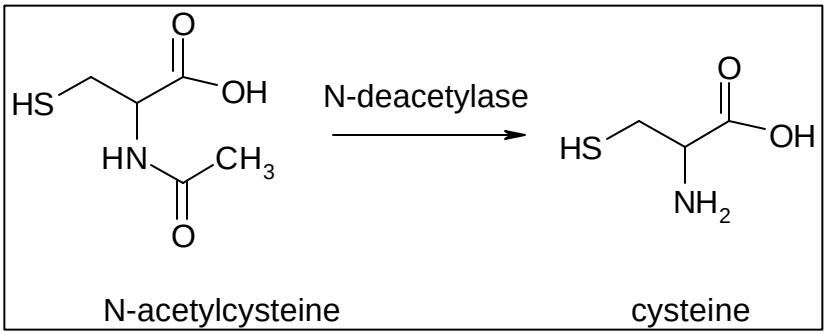

Figure 1: N-Deacetylation of NAC Gives Cysteine

(as seen in Figure 1) to create cysteine [8]. Cysteine is then used in the production of the tripeptide thiol GSH. (For a detailed description of GSH biochemistry, please see the excellent review in this volume.)

There is ample evidence that NAC can be deacetylated by animal cells. NAC can be deacetylated by isolated hepatocytes [9], rat intestinal cells [10], homogenates of rat intestine [11], homogenates of rat lung and liver [2], and homogenates of human liver [2].

When NAC is oxidized, it forms the disulfide RSSR [5, 6]. See Figure 2.

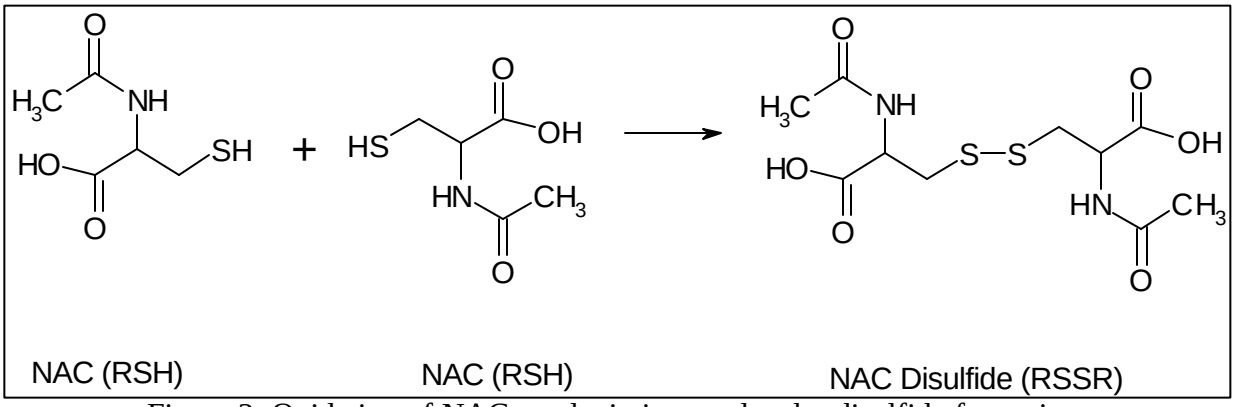

Figure 2: Oxidation of NAC results in intermolecular disulfide formation.

N-Acetylcysteine and Hypochlorous Acid

N-acetylcysteine is a very good scavenger of hypochlorous acid. In one study, NAC prevented HOCl-treated guinea pig tracheal smooth muscle from contracting [12]. In another, a_1-antiproteinase, which is frequently damaged by HOCl, was protected against inactivation by NAC. This study concluded that NAC is a very powerful HOCl scavenger, although a rate constant was not calculated [5]. This ability of NAC to scavenge HOCl may be of particular importance in therapeutic protection of the lung. Inflammation causes macrophages to release HOCl, which inactivates a_1-antiproteinase (a serine protease inhibitor), resulting in a lack of

inhibition of elastase. Elastase, a serine protease, hydrolyzes lung elastin, leading eventually to emphysema [5].

N-Acetylcysteine and Hydroxyl Radical

Thiols are usually good scavengers of $HO^{\bullet}$, and NAC is no different. The rate constant at pH 7.0 is 1.36 x 10^{10} $M^{-1}s^{-1}$, very near diffusion-controlled [5]. Thus, a very important therapeutic and experimental role for NAC could be in the scavenging of $HO^{\bullet}$.

N-Acetylcysteine and Hydrogen Peroxide

N-acetylcysteine reacts with H_2O_2, although very slowly. Initial attempts to establish a rate constant for the reaction utilized a horseradish peroxidase-based (HRP) system, but NAC interfered with the studies because thiols are HRP substrates. However, when the reaction was examined by looking at the loss of the –SH group using 2-nitrobenzoic acid, the Aruoma group was able to calculate a rate constant of 0.85 $M^{-1}s^{-1}$ for the scavenging of H_2O_2 by NAC [5]. Thus, although NAC does react with H_2O_2, it is unlikely that this extremely slow reaction is of much biological significance.

The reaction of NAC (RSH) with H_2O_2 has been broken down into two steps. Raman and infrared spectroscopy have confirmed that NAC first forms a complex with hydrogen peroxide, as seen in equation 1. The exact stoichiometry of this reaction depends upon the concentration of NAC. Then, this complex is converted into NAC disulfide (RSSR) [6]. Although the two rate

$$2RSH + H_2O_2 \underset{k = 0.9\ s^{-1}}{\overset{k = 90\ M^{-2}\ s^{-1}}{\rightleftharpoons}} [RSH\ldots H_2O_2\ldots RSH] \xrightarrow{k = 0.03\ s^{-1}} RSSR + 2\ H_2O \qquad (1)$$

constants for this reaction differ ($k=0.85\ M^{-1}\ s^{-1}$ and $k=0.03\ s^{-1}$), the difference is not very significant. Most importantly, both groups agree that the reaction of hydrogen peroxide with NAC is an extremely slow reaction.

N-Acetylcysteine and Superoxide

The reaction rate of thiols and superoxide is a somewhat disputed matter. One group reported that $O_2^{\bullet-}$ and cysteine reacted extremely slowly [13], while another gave a rate constant of $2.7 \times 10^6\ M^{-1}s^{-1}$ [14]. The reaction rate of NAC with $O_2^{\bullet-}$, on the other hand, has only recently been studied successfully. This study established a rate constant of $68\ M^{-1}s^{-1}$ at pH=7.4, suggesting that the reaction of $O_2^{\bullet-}$ with NAC is insignificant at physiologic concentrations of $O_2^{\bullet-}$ [6].

The following mechanism has been proposed by Benrahmoune *et al.* to describe the reaction of NAC with superoxide [6].

$$RSH + O_2^{\bullet-} \rightleftharpoons [RS(OOH)^{\bullet-}] \quad (2)$$

$$[RS(OOH)^{\bullet-}] \longrightarrow RSO^{\bullet} + OH^- \quad (3)$$

$$RSO^{\bullet} + O_2^{\bullet-} \xrightarrow{H^+} RSOH + O_2 \quad (4)$$

$$RSOH + RSH \longrightarrow RSSR + H_2O \quad (5)$$

Since the formation of H_2O_2 was significantly lower than the formation of RSSR, this study concluded that the initial reaction of NAC with superoxide was unlikely to involve hydrogen abstraction by superoxide from NAC, as shown in equation 6 [6].

$$RSH + O_2^{\bullet-} \longrightarrow RS^{\bullet} + H_2O_2 \quad (6)$$

A background reaction that will compete with reaction 4 is [6]:

$$RSO^{\bullet} + RSH \longrightarrow RSOH + RS^{\bullet} \quad (7)$$

This may occur to a small extent, as RSH is an extremely reducing species. However, the rate constant for reaction 7 will be slower than that of reaction 4, as the latter is a radical-radical reaction. In addition, in the Benrahmoune *et al.* paper, they reasoned that RSO_2H should be formed, according to reactions 8 and 9. However, they were unable to find RSO_2H in their end products, so concluded that the reaction of $RSO^{\bullet}$ with RSH is insignificant [6].

$$RS^{\bullet} + O_2 \rightleftharpoons RSOO^{\bullet} \quad (8)$$

$$RSOO^{\bullet} + RSH \longrightarrow RSO_2H + RS^{\bullet} \quad (9)$$

NAC in Action: Mechanism of Acetaminophen Detoxification

When acetaminophen is ingested, our body metabolizes about 5 to 10% of it to N-acetylbenzochionimine, a substance which reacts with thiols in the liver. When an overdose is taken, hepatic glutathione is oxidized to glutathione disulfide. If the liver's supply of GSH is exceeded, the liver will become necrotic [8]. Administration of GSH is not particularly useful because it is not taken up by cells [15]. Thus, the usefulness of NAC as a treatment for acetaminophen poisoning becomes evident. N-acetylcysteine is both a precursor for GSH [8] and a powerful antioxidant [5, 6]. Also, about 70% of NAC is metabolized in the liver, so it is in the right place at the right time to prevent acetaminophen toxicity [16].

Methods for Detecting and Measuring N-Acetylcysteine

Detecting NAC in a biological setting has been a difficult problem to overcome for researchers. NAC has few physical properties that allow its detection. Also, it can easily be

oxidized to its disulfide form, which can produce artifacts. Also, since biological systems contain other low molecular weight thiols such as cysteine and glutathione, it can be difficult to distinguish between NAC and these other species, which have similar physical and chemical properties to NAC [2]. One way this has been overcome is through reversed-phase high-performance liquid chromatography (HPLC) methods, which trap reduced NAC as a stable, detectable adduct [2, 17].

One particularly rapid and sensitive method for NAC detection uses N-(1-pyrenyl)maleimide (NPM) in an HPLC system. See Figure 3 below. In this system, NPM forms a fluorescent adduct with NAC, allowing the product to be easily detected and quantified. The main advantages of this system are that it is a very fast assay, can be

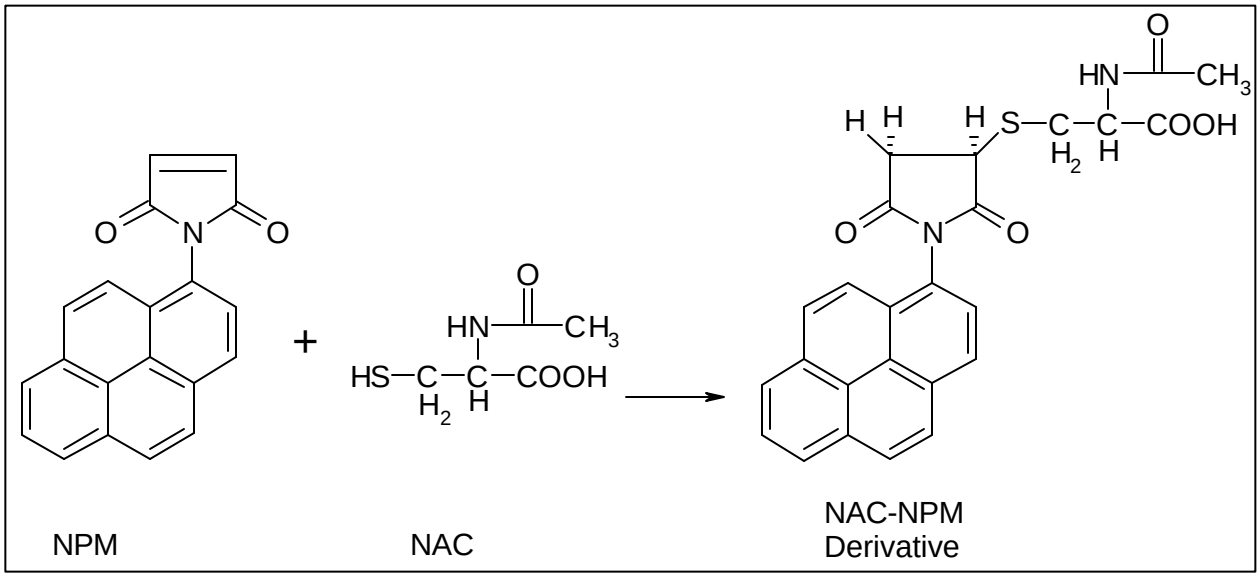

Figure 3: NPM is used to detect NAC in biological systems with an HPLC system. Adapted from [17].

automated, has a range of detection from 8 nM to 2.5 µM, is highly reproducible, and recovers most of the NAC from a biological system. Oxidized NAC can also be reduced by dithiothreotol prior to the assay, resulting in a more accurate picture of total cellular NAC [17].

Summary

N-acetylcysteine is a very small but powerful antioxidant molecule. It has been used safely in the treatment of congestive and obstructive lung diseases for many years and has recently become the treatment of choice for acetaminophen overdose. Its power as a detoxifying molecule is seen in its extremely fast reaction rates with both hypochlorous acid and hydroxyl radical. Reactions with superoxide and hydrogen peroxide are also possible, although these reactions take place at an almost insignificant rate. In addition to its own antioxidant properties, NAC also enhances the GSH pool—both as a glutathione precursor and by enhancing glutathione reductase activity. Thus NAC has been and will continue to be a very safe and effective therapeutic agent.

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