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N-Acetyl-L-Cysteine

(NAC)

by

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Abbreviations

DHL, dihydrolipoic acid
DMPO, 5,5-dimethyl-1-pyrroline-*N*-oxide
DTT, dithiothreitol
EPR, electron paramagnetic resonance
GSH, glutathione
HIV, human immunodeficiency virus
$HO^{\bullet}$, hydroxyl radical
LC-UV-MS, liquid chromatography-ultraviolet detection-mass spectrophotometry
NPM, N-(1-pyrenyl)maleimide
RP-HPLC, revered-phase high performance liquid chromatography
ROS, reactive oxygen species
SCN^{-}, thiocyanate ion
$O_2^{\bullet -}$, superoxide

Table of Contents **Page**

Abstract

N-Acetyl-L-Cysteine (NAC) is a low-molecular weight compound and an excellent source of sulfhydryl (SH) groups. NAC functions as a precursor in GSH synthesis and a thiol antioxidant. Here, we review the properties and mechanism of action of NAC, as well as effective methods for its detection. NAC acts as a scavenger of hydroxyl radical, superoxide and hydrogen peroxide, though it reacts relatively slowly with superoxide and hydrogen peroxide. Initially, NAC was used as a mucolytic agent but now it is studied and utilized in diseases characterized by decreased glutathione (GSH) levels or oxidative stress, such as cancer, heart disease and HIV infection. Methods for detecting and measuring NAC have been developed and include such assays as reversed-phase high-performance liquid chromatography (RP-HPLC) and liquid chromatography-ultraviolet detection-mass spectrophotometry (LC-UV-MS).

Introduction

N-Acetyl-L-Cysteine (NAC) is a low-molecular weight thiol compound (MW = 163.20) with chemical formula $C_5H_9NO_3S$ [1]. It is also an acetylated form of the amino acid L-cysteine (**Figure 1**) [1,2]. NAC is a weak acid, with a p*K*a of 3.24 [3].

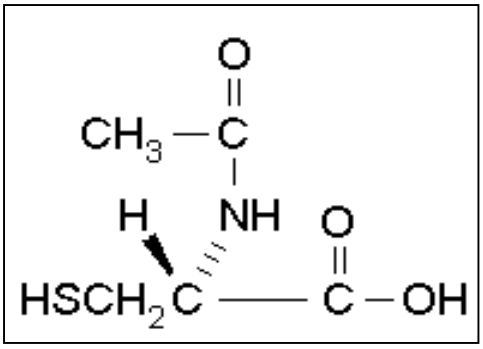

Figure 1. Structure of NAC [3].

NAC has been used in experimental and clinical applications, primarily as a mucolytic agent, since the 1950s. Indeed its benefit as a mucolytic agent was demonstrated in lung diseases characterized by hypersecretion of respiratory mucus, such as cystic fibrosis and chronic bronchitis [4]. Then, in the early 1970s, NAC was administered intravenously to Swiss mice whose various tissues were then examined for protein and nonprotein sulfhydryl content [5]. These studies demonstrated that tissue sulfhydryl content was dramatically higher in the presence of NAC, suggesting that NAC is an excellent source of sulfhydryl groups [5]. More recently, NAC has been used as a therapeutic agent in the treatment of acetaminophen overdose [1]. NAC has also been proven beneficial in the treatment of cancer, heart disease and human immunodeficiency virus (HIV) infection [2]. NAC is rapidly absorbed following an oral administration but only a small percentage of intact NAC appears in plasma and tissue [2]. Low oral bioavailability of NAC suggests that NAC is quickly metabolized into other compounds,

such as cysteine. Thus, only four to ten percent of intact NAC is bioavailable in the plasma and subsequently in tissue [2].

Mechanism of Action

Most of the benefits observed in the presence of orally administered NAC are believed to occur due to its sulfhydryl chemistry. Indeed, as a source of sulfhydryl (SH) groups, NAC has the ability to stimulate GSH synthesis, increase glutathione-S-transferase activity and scavenge reactive oxygen species (ROS) [2].

A. Precursor in GSH synthesis

N-deacetylation of NAC results in the production of the amino acid cysteine (**Figure 2**) [2]. Cysteine is one of the three amino acids used in the biosynthesis of a major cellular antioxidant glutathione (GSH). The other two amino acids used to generate the tripeptide GSH are glutamate and glycine [6]. Thus, by increasing cysteine levels through deacetylation, NAC is theorized to act as a precursor in GSH synthesis [2]. The ability of NAC to become deacetylated in animal cells has been demonstrated in rat intestinal cells [7] and rat hepatocytes [8], as well as homogenates of rat and human liver [4].

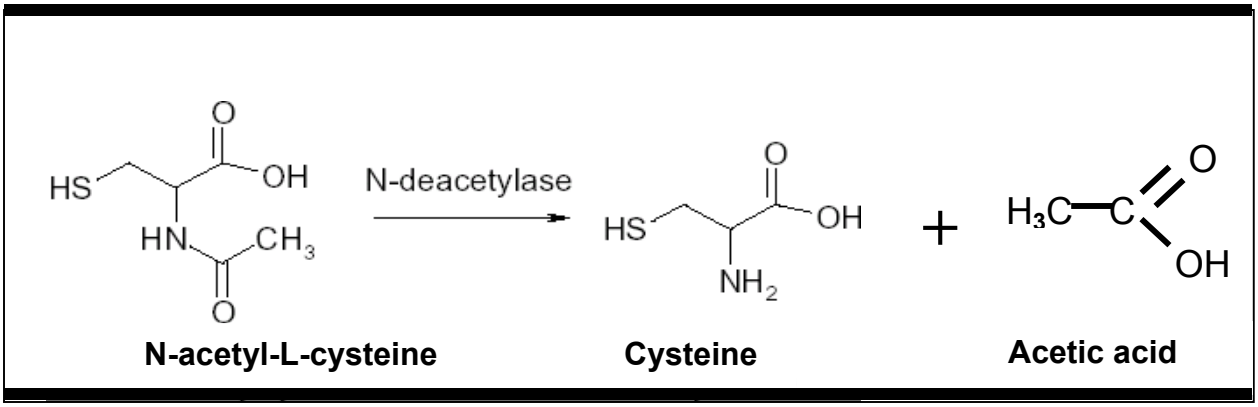

Figure 2. Deacetylation of NAC generates cysteine [2].

B. Antioxidant

NAC is also considered to be an effective thiol antioxidant because it can directly act on reactive oxygen species, such as hydroxyl radical ($HO^{\bullet}$) and superoxide ($O_2^{\bullet-}$).

I. NAC and Hydroxyl Radical

NAC is a very good scavenger of hydroxyl radical, $HO^{\bullet}$. At pH 7.0, the rate constant for the reaction between NAC and hydroxyl radical is 1.36×10^{10} M^{-1} s^{-1} [9]. The rate constant for this reaction was determined using competitive binding of NAC and SCN^- to hydroxyl radical. Measurement of the rate constant involved several steps: A) radiolysis of water to produce hydroxyl radical (Reaction 1), B) saturation with nitrous oxide to convert the aqueous electrons to hydroxyl radical (Reaction 2), C) reaction with thiocyanate ion (SCN^-) to generate $(SCN)_2^-$ radical (Reaction 3) and, finally, D) measuring the reaction rate using the competition kinetics between NAC and SCN^- for hydroxyl radical (Reaction 4 and 5) [9]. When compared to other ROS, the reaction of NAC with hydroxyl radical is among the fastest, nearing diffusion limit [9].

$H_2O \rightarrow HO^{\bullet}, e^-_{(aq)}, H^{\bullet}, H_2O_2, H_2$ (Reaction 1)

$e^-_{(aq)} + N_2O + H_2O \rightarrow HO^{\bullet} + OH^- + N_2$ (Reaction 2)

$HO^{\bullet} + SCN^- \rightarrow HOSCN^-$ (Reaction 3)

$HO^{\bullet} + NAC\text{-}SH \rightarrow NAC\text{-}S^{\bullet} + H_2O$ (Reaction 4)

$2\ NAC\text{-}S^{\bullet} \rightarrow NAC\text{-}S\text{-}S\text{-}NAC$ (Reaction 5)

II. NAC and Superoxide

NAC also readily reacts with superoxide ($O_2^{\bullet-}$) as shown in the following reaction [10]:

$RSH + O_2^{\bullet-} \leftrightarrows [RS(OOH)^{\bullet-}]$ (Reaction 6)

$[RS(OOH)^{\bullet-}] \rightarrow RSO + OH$ (Reaction 7)

H^+

$RSO^{\bullet} + O_2^{\bullet-} \rightarrow RSOH + O_2$ (Reaction 8)

$RSOH + RSH \rightarrow RSSR + H_2O$ (Reaction 9)

Initially, the rate constant for the reaction of thiols with superoxide was calculated using electron paramagnetic resonance (EPR) method, where superoxide radials were spin trapped with 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) and then reacted with various thiols, including GSH and dihydrolipoic acid (DHL) [11]. This study reported the rate constant value of $1\text{-}5 \times 10^5$ M^{-1} s^{-1} [11]. However, Winterbourn *et al*. [12] recently found that the relative reactivity of thiols is inversely related to the p*K* value of the thiol group [12], suggesting that $1\text{-}5 \times 10^5$ M^{-1} s^{-1} rate constant value is too high. Winterbourn *et al*. [12] estimated that the rate constant for the reaction of NAC with superoxide is between 30-1000 M^{-1} s^{-1} because at physiologic pH NAC would be weakly reactive [12]. Consistent with the report by Winterbourn and colleagues, Benrahmoune *et al*. [10] determined that the rate constant for the reaction of NAC with superoxide at pH 7.4 was 68 M^{-1} s^{-1}. This result suggested that such reaction is insignificant at physiologic concentrations of superoxide [10].

Another reaction is in competition with Reaction 8 [10]:

$RSO^{\bullet} + RSH \rightarrow RSOH + RS^{\bullet}$ (Reaction 10)

$RSO^{\bullet}$ is considered to be a moderate oxidizing radical so hydrogen atom abstraction reaction from a thiol is less likely to occur (Reaction 10) than an electron transfer reaction. Based on the following two reactions, Benrahmoune *et al*. [10] suggested that RSO_2H should be formed:

$RS^{\bullet} + O_2 \leftrightarrows RSOO^{\bullet}$ (Reaction 11)

$RSOO^{\bullet} + RSH \rightarrow RSO_2H + RS^{\bullet}$ (Reaction 12)

From their end-product analysis, however, Benrahmoune *et al*. [10] concluded that RSO_2H is not formed, suggesting that Reaction 10 only has minor importance. In addition, Benrahmoune *et al*.

[10] concluded that hydrogen peroxide is not a product of the reaction between NAC and superoxide because the reaction of $O_2^{\bullet -}$ with RSH failed to proceed via the abstraction of hydrogen atom from RSH (Reaction 13) [10]:

$$RSH + O_2^{\bullet -} \rightarrow RS^{\bullet} + H_2O_2 \qquad \text{(Reaction 13)}$$

III. NAC and Hydrogen Peroxide

The reaction of NAC with hydrogen peroxide proceeds very slowly and it involves two steps. In the first step, NAC forms a complex with hydrogen peroxide as shown in Reaction 14. Then, in the second step, this complex is converted to disulfide-bonded form of NAC, RSSR (Reaction 15). The *k* values for Reaction 14 and 15 were computed by examining the loss of sulfydryl groups using 2-nitrobenzoic acid [9]:

$$2RSH + H_2O_2 \underset{k_{-13} = 0.90\ s^{-1}}{\overset{k_{13} = 90\ M^{-1}\ s^{-1}}{\leftrightarrows}} [RSH\ldots\ H_2O_2\ldots RSH] \qquad \text{(Reaction 14)}$$

$$[RSH\ldots\ H_2O_2\ldots RSH] \overset{k_{14} = 0.03\ s^{-1}}{\rightarrow} RSSR + 2H_2O \qquad \text{(Reaction 15)}$$

Because the rate constant for Reaction 14 is so slow, it is considered the rate-limiting step in the reaction between NAC and hydrogen peroxide.

Measuring and Detecting NAC

Methods for detection of NAC in a biological system have proved challenging to develop because biological systems contain other low molecular weight compounds, such as glutathione and cysteine, with similar physical and chemical properties to NAC. Furthermore, NAC is easily oxidized to its disulfide form, which can produce artifacts [4]. However, effective methods have been developed for detecting and quantifying NAC some of which include reversed-phase high-

performance liquid chromatography (RP-HPLC) and liquid chromatography-ultraviolet detection-mass spectrophotometry (LC-UV-MS).

A. Reversed-phase high-performance liquid chromatography (RP-HPLC)

In this assay, thiols like NAC are converted to their S-nitroso derivatives by excess of nitrite, followed by their cation-pairing RP-HPLC with detection at 333 nm. The recovery rate of NAC using this method was 99.6%. This method has been successfully applied in a pharmacokinetic study on NAC delivered orally to healthy volunteers [13].

One particularly sensitive method for detecting NAC uses reagent N-(1-pyrenyl)maleimide (NPM) in an HPLC system (**Figure 3**). In this assay, NPM binds to NAC to form a fluorescent adduct, which can then be easily detected and quantified. Oxidized NAC can be reduced by dithiothreitol (DTT) prior to the assay in order to allow for more accurate picture of the total cellular NAC content [14]. This NPM-based HPLC system has numerous advantages for detecting NAC. It is highly reproducible, rapid and automated assay with a detection range of 8 nM to 2.5 μM [14].

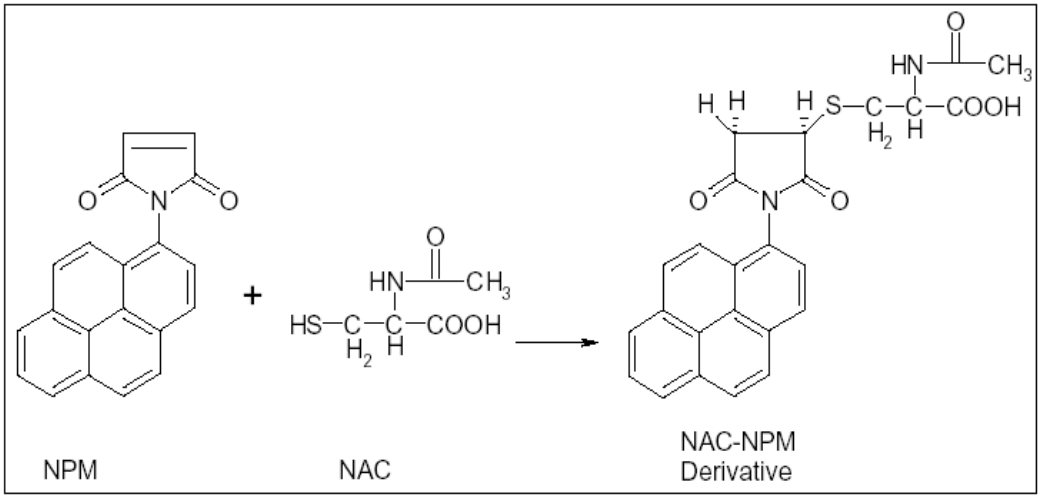

Figure 3. NPM-based HPLC assay is used to detect NAC in biological systems [14].

Liquid chromatography-ultraviolet detection-mass spectrophotometry (LC-UV-MS)

This method is based on on-line LC-UV-MS system, where the stability of the thiol-based NAC is maintained by the acidic pH of the LC mobile phase. UV detection prevents ion-source overloading effect due to high NAC concentrations, while MS separates and quantifies the impurities. This method has proven to be effective in terms of stability, linearity and accuracy [15].

Summary

NAC is a small, thiol-based antioxidant. It readily reacts with hydroxyl radical, as well as superoxide and hydrogen peroxide but these two latter reactions occur at a significantly lower rate. In addition to its role as a ROS scavenger, NAC is able to act as a precursor in GSH biosynthesis, mucolytic agent and detoxification promoter. As such, NAC has been used effectively in treatment of various diseases, including respiratory illnesses, cancer and HIV infection.

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