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N-Acetyl-L-Cysteine

(NAC)

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Abbreviations:

DMPO: 5,5'-Dimethyl-1-pyrrolidine N-oxide;
DTNB: Dithiobis (2-nitrobenzoic acid);
GSH: Glutathione;
HPLC: High performance liquid chromatography;
NAC: N-Acetyl-L-Cysteine;
NBT: Nitroblue tetrazolium;
RP-HPLC: Reversed phase high performance liquid chromatography

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I. Abstract

N-acetylcysteine is a thiol compound with chemical formula $C_5H_9NO_3S$ and a molecular weight of 163.2 NAC was initially used as a mucolytic agent, but is now being recognized and utilized as a therapeutic drug for diseases associated with decreased gluthathione levels or oxidative stress such as HIV infection, heart disease, and cancer. Although the exact mechanism of action is not yet very clear yet, NAC has been speculated to exert its effects *via* its sulfhydryl chemistry as also by its reactivity to reactive oxygen species. Hence this paper reviews the properties of NAC, its mechanism of action, reactivity and detection methods.

II. Introduction

N-acetyl L-cysteine (NAC) is a small molecular weight thiol compound (M.W 163). It is an acetylated form of the amino acid L-cysteine. As early as 1970, NAC was shown as a source of sulfhydryl groups [1], in experiments, where NAC was given intravenously in Swiss mice and the protein and nonprotein sulfhydryl content of various tissues fractions was analyzed. The tissue protein sulfhydryl content was significantly higher with NAC. In addition to being a sulfhydryl source, NAC is also known as a thiol antioxidant due to its reactivity with oxidant species like $O_2^{\bullet -}$, H_2O_2, and $\bullet OH$ [2].

Initially used as a mucolytic agent [3], NAC now has proven to be a very important therapeutic agent in treatment of paracetomol poisoning [4], and as a cardioprotector against doxorubicin. It is also very beneficial in treatment of human immunodeficiency virus infection [5] and heart diseases. Presently with its use in cancer treatment, NAC is one of the most promising chemopreventive agents. The LD50 of NAC is 7888 mg/kg in mice and greater than 6000 mg/kg in rats following oral administration [6]. Even though it is rapidly absorbed, the bioavailability of intact NAC is only about four to ten percent. Most of it gets incorporated into proteins and in the formation of other metabolites.

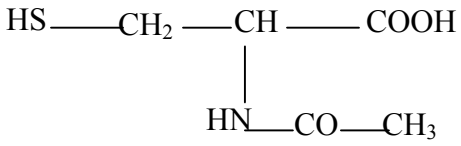

Figure1. Chemical structure of N-acetyl-L-cysteine [11]

III. Mechanism of Action

III.1. Precursor of GSH synthesis.

Inspite of the wide spectrum of applications, very little is known about the exact mechanism of NAC action. NAC has long been suggested to exert its effect by increasing cysteine levels, and thereby act as a precursor of glutathione synthesis [6]. As a cource of –SH groups, NAC can stimulate GSH synthesis and also enhance glutathione-S-transferease activity.

III.2 Thiol Antioxidant:

NAC is also called a thiol antioxidant, due to its reactivity towards reactive oxygen species, H_2O_2, and •OH.

III.2.a Reaction with Superoxide ($O_2^{\bullet -}$):

$$NAC\text{-}SH + O_2^{\bullet -} + H^+ \longrightarrow NAC\text{-}S + H_2O_2 \qquad \text{(Reaction 1)}$$

Like other thiols, NAC reacts with superoxide and can cause formation of hydrogen peroxide (Reaction 1) [8]. Early studies of NAC reactions with superoxide yielded uncertain results. Most of them were concluded to be invalid due to the assays used like the cytochrome c or NBT assay. Both the assays showed that NAC reacts with superoxide with a rate constant less than 10^3 $M^{-1}s^{-1}$.However in this assay possible regeneration of superoxide and could result in a wrong interpretationof the reaction rate. *k* values of 1-5 x 10^5 $M^{-1}s^{-1}$ were calculated by spin trapping superoxide radicals with DMPO and reacting them with different thiols including NAC [7]. These rate constants are considered to be too high. Recent reports suggest that the relative reactivity of thiols is inversely proportional to the pK of the thiol group [8]. Based on this hypothesis rate

constant for NAC was estimated to be in the 30-1000 $M^{-1}s^{-1}$ range since at pH 7.4, NAC (pk 9.5) would be weakly reactive. In agreement with this, using radiolysis experiments, a rate constant of 68 ± 6 $M^{-1}s^{-1}$ was computed [9].

III.2.b. Reaction with hydrogen peroxide (H_2O_2):

$$NAC\text{-}S^- + H_2O_2 + H^+ \longrightarrow NAC\text{-}SOH + H_2O_2 \quad \text{(Reaction 2)}$$

$$NAC\text{-}SOH + NAC\text{-}SH \longrightarrow NAC\text{-}SS\text{-}NAC + H_2O \quad \text{(Reaction 3)}$$

H_2O_2 reacts very slowly with NAC, producing the sulfenic acid form (Reaction 2). In the presence of excess NAC, the sulfenic acid combines with reduced NAC to yield disulfide form of NAC (Reaction 3). Initial experiments aimed at investigating the reaction rates of NAC and H_2O_2 involved peroxidase. It was later found that NAC is one of the substrates for horseradish peroxidase. Hence another assay was used to calculate rate constants of NAC with H_2O_2, which involved determining the loss of –SH groups using DTNB. This assay calculated a second-order rate constant of 0.85 $M^{-1}s^{-1}$ (±10%). Taking into account the very low concentration of H_2O_2 *in vivo*, this reaction may not be very significant [2].

III.2.c Reaction with hydroxyl radical (·OH)

NAC is a very good ·OH radical scavenger. The rate of reaction is calculated by first generating hydroxyl radical by radiolysis of water (Reaction 4), followed by saturation with nitrous oxide that converts the aqueous electrons to hydroxyl radicals (Reaction 5).

$H_2O \longrightarrow \bullet OH, e^-_{(aq)}, H\bullet, H_2O_2, H_2$ (Reaction 4)

$e^-_{(aq)} + N_2O + H_2O \longrightarrow \bullet OH + OH^- + N_2$ (Reaction 5)

$\bullet OH + SCN^- \longrightarrow HOSCN^-$ (Reaction 6)

$\bullet OH + NAC \longrightarrow HONAC$ (Reaction 7)

The hydroxyl radical is then treated with thiocyanate ion to give the radical: $\bullet (SCN)_2^-$. NAC reaction rate with hydroxyl radical (Reaction 7) is measured using competition kinetics between NAC and SCN^- , for $\bullet OH$. Aruoma *et al* calculated this rate to be 1.4 x 10^{10} $M^{-1}s^{-1}$ [2]. Hence compared to other reactive oxygen species, the reaction of NAC with hydroxyl radical is the fastest, approaching diffusion limits.

Another scavenger target of NAC is hypochlorous acid (HOCL). HOCL is made by the neutrophil enzyme myeloperoxidase, by oxidizing Cl- ions sites of inflammation. HOCl attacks α-proteinase, which inhibits the serine proteases like elastase. Hence in order to study the scavenging action of NAC, it was added to reaction containing HOCl, α-proteinase and elastase. About 96 uM of NAC was found to protect α-proteinase from HOCl.

Detection of NAC

Numerous methods have been developed to detect and quantitate levels on NAC in aqueous solutions as well as in biological fluids. Most of the methods involve derivatising the molecule, suitable for detection by spectrophotometry, flurometry or chromatography. Some of the techniques used are:

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

In this method thiols are converted to their S-nitroso derivatives by excess of nitrite treatment. NAC levels are then detected RP-HPLC and UV detection [10]. RP-HPLC is a type of HPLC that uses a hydrophobic stationary phase, which increases efficiency of detecting molecules like amino acids and carbohydrates Figure 1 shows representative RP-HPLC choromatograms obtained from plasma.

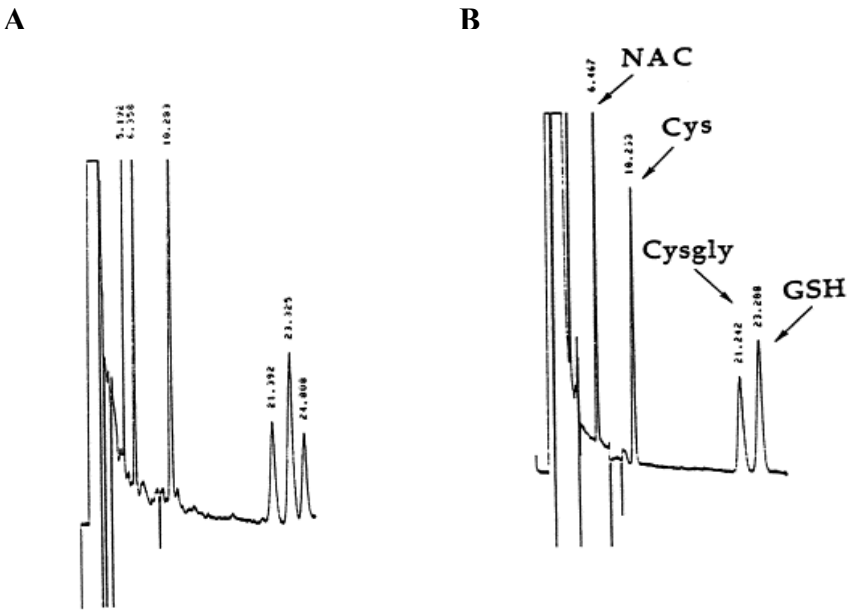

Fig 1(A) Representative chromatogram from RP-HPLC analysis of plasma samples following 10 uM NAC. (B) Reference mixture-containing 10 uM NAC, Cys, Cysgly and GSH in 50 mM phosphate buffer (pH 7.4).

2) Liquid chromatography-UV-mass spectrometry (LC-UV-MS).

This is a relatively new method of quantitative analysis, which is usually used in the detection of impurities present along with NAC, in pharmaceutical formulations (11).

3) Spectroscopic Studies

Using infrared and Raman scattering, studies have been carried out, to observe NAC (RSH) and NAC (RSSR) forms complexed to H_2O_2 and investigate mechanism of this redox reaction (12).

Summary

NAC is a small molecular weight, cell permeable thiol that is a variant of the amino acid cysteine. In addition to being a precursor of GSH synthesis, NAC also functions as a free radical scavenger. Initially, NAC clinically used as a mucolytic agent, is now being tested widely in the treatment of diseases ranging from HIV infection to cancer. Due to the low toxicity coupled with ease of administration, NAC could have great potential as a therapeutic drug for many diseases linked to oxidative stress.

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