Population, Acid−Base, and Redox Properties of *N*-Acetylcysteine Conformers

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Rotamers of *N*-acetyl-L-cysteine (NAC, the most popular mucolytic drug) are characterized in terms of populations, site- and conformer-specific acid−base properties, reducing strength, and molecular pharmacology. A new, general relationship between the bulk- and rotamer-specific basicities is introduced. NAC at high pH predominantly exists in a trans thiolate−carboxylate rotameric form, whereas protonation promotes the occurrence of intramolecular hydrogen bond-forming isomers. Distribution curves of the rotamers are depicted as a function of pH. Rotamer-dependent thiolate basicities differ by up to 0.5 log *k* units. Carboxylate basicities show slight conformation-dependence only. The membrane-penetrating capabilities from various compartments of the body are assessed on the basis of the pH-dependent charge of the molecule. The thiol−disulfide half-cell potential is calculated, using the correlation between the thiolate basicity and oxidizability. The oxidation−reduction properties of NAC are compared to those of other biological thiols in their definite microscopic forms. The pharmacokinetic behavior is interpreted in terms of the physicochemical parameters, providing molecular/submolecular explanation for several therapeutic properties of NAC.

Introduction

N-Acetyl-L-cysteine (NAC) is a molecule of extremely fast medicinal career and wide therapeutic profile. To date, at least 12 distinct biological activities have been attributed to NAC.[1,2] In view of the fact that NAC is one of the smallest drug molecules, with a total of 19 atoms, the above pharmacological versatility is undoubtedly unique, indicating that the background of its biological complexity must be sought at the submolecular level.

The early indication for NAC was to lessen the toxic effects of paracetamol overdosage.[1,3] Today, however, NAC is the most widely used mucolytic agent worldwide.[1,4] Further indications for its use include rheumatoid arthritis,[1] plasma hyperlipoproteinaemia,[5] and adult respiratory distress syndrome.[6] NAC has also been reported to support repair processes after cytotoxic and radioactive damages in cancer therapy,[1,7] to remove atherosclerotic plaques,[8] to increase the survival rate of hippocampal neurons[9] and ischaemic tissues,[10] and to improve the bacterial clearance.[11]

Its mode of action has generally been assumed to be related to the thiol (or thiolate) site, as the key moiety of the molecule. The reported thiolate-involved mechanisms are free radical scavenging,[9−11] rupture of disulfide bonds in cross-linked mucous proteins,[1,4] metal complex formation,[2] and acting as part of the glutathione (GSH/GSSG) redox system.[3,7]

The great medicinal importance has generated several theories on its in vivo behavior. Some papers claim that NAC penetrates the cell membranes.[5,12] Some others[7,13] deny that, on the basis of its poor (5%) bioavailability[13,14] and very low alveolar concentration after oral administration.[13] Its role as glutathione precursor has been both hypothesized[3,7] and refuted by pharmacokinetic studies.[15−16]

The chemical properties of NAC have been characterized in terms of metal complexation constants,[17,18] metal ion-promoted amide deprotonation,[19] crystal structure,[20] analytical stability in various pharmaceutical products,[21] and protonation constants, determined by pH−potentiometry,[17,22] UV−pH titration,[23] and kinetic methods.[24]

No paper appeared, however, on two crucial properties of NAC that govern its viability and activity in biological media. Namely, its solution conformation and the related protonation state- and medium-dependent rotational flexibility that influence the membrane-penetration and binding propensities have not been reported.

Also, no data can be found on the oxidation−reduction potential, the key parameter in thiol−disulfide equilibrium and free-radical-scavenging processes.

Characterization of solution conformation in terms of rotamer populations for molecules of ABX spin systems has become possible through the measurement of ^{1}H NMR three-bond coupling constants and an appropriate evaluation procedure. Thus, rotamer populations have been determined[25−27] for various amino acids and related compounds. These studies also revealed subtle differences in the site- and conformation-specific basicities of the coexisting, fast interconverting rotamers. The quantitation of thiol−disulfide oxidation−reduction potentials is a difficult task and can be achieved for limited kinds of compounds only. The formation of stable metal−thiolate complexes at electrode surfaces and the concomitant electrochemical irreversibility prevent the use of standard voltammetric methods.[28] Reaction mixture analysis of thiol−disulfide equilibrium systems by spectroscopic techniques[29,30] is a substitute choice, which

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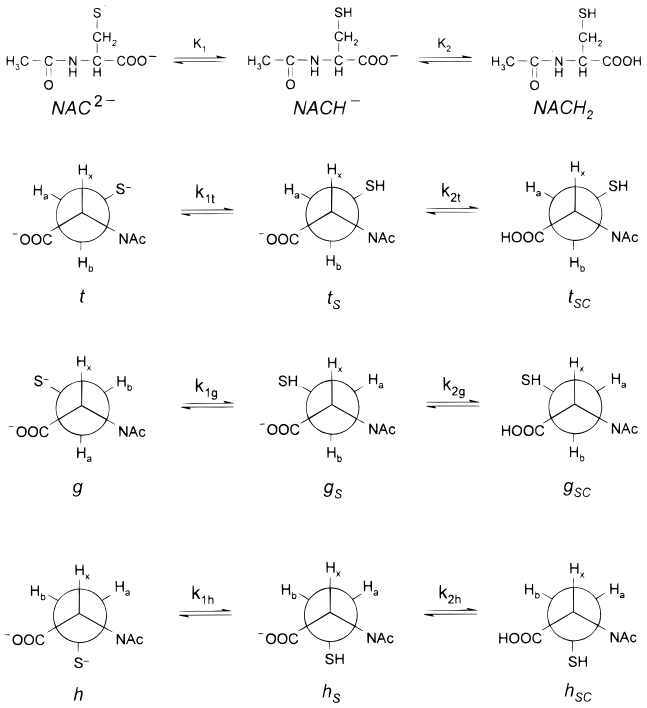

Figure 1. Protonation scheme of *N*-acetyl-L-cysteine and its rotamers.

is usually seriously hampered by the high spectroscopic similarity of the analytes, especially in the cases of large molecules.

The recently observed relationship[31] between the basicity and oxidizability of thiolates is a significant progress in the biological chemistry of these compounds, providing a means to characterize the thiol−disulfide chemistry, even for compounds with more than one thiolate site.[32]

Here we report the determination of rotamer concentrations and site- and conformation-specific basicities for the unseparable, coexisting NAC rotamers. A new, general relationship between the bulk and rotamer-specific basicities is introduced. The membrane-penetrating capabilities from various compartments of the body are assessed on the basis of the pH-dependent charge of the molecule. The thiol−disulfide half-cell potential is calculated and compared to those of other biological thiols in their definite microscopic forms. These parameters allow the interpretation of the therapeutic behavior of NAC at the submolecular level, providing a picture consistent with the pharmacokinetic and biochemical findings.

Results and Discussion

Rotamer Populations. Figure 1 shows the bulk and rotamer-specific protonation equlibria and constants. Abbreviations t, g, h, t_S, g_S, h_S, t_{SC}, g_{SC}, and h_{SC} stand for the corresponding rotamer-specific forms, where subscript(s) (if any) indicate the protonated site(s).

The basicity of the thiolate site is well-known to be significantly higher than that of the carboxylate. Thus, the process of protonation takes place in well-separated steps in distinct pH ranges, where the nonprotonated, monoprotonated, and diprotonated species occur overwhelmingly.

Table 1. ^{1}H NMR Data of *N*-Acetylcysteine in 90% H_2O−10% D_2O Solution

	chemical shift, ppm				observed coupling, Hz		
pH	H_a	H_b	H_x	H_3C-CO-	$^3J_{Xa}$	$^3J_{Xb}$	$^2J_{ab}$
0.98	2.99	2.97	4.64	2.07	4.5	6.8	14.2
7.13	2.92	2.90	4.37	2.06	4.1	6.6	13.9
12.12	2.91	2.66	3.92	2.05	3.7	9.6	13.2

The three-bond, observed ^{1}H−^{1}H coupling constants at pH ranges above 12, near 7, and below 1 reflect rotamer populations virtually at one single protonation stage of the molecule. Taking the example of the nonprotonated rotamers, equations (1−3) hold for the $^3J_{AX}$ and $^3J_{BX}$ observed coupling values:

$$^3J_{AX} = f_t J_G + f_g J_T + f_h J_G \quad (1)$$

$$^3J_{BX} = f_t J_T + f_g J_G + f_h J_G \quad (2)$$

$$f_t + f_g + f_h = 1 \quad (3)$$

where f_t, f_g, f_h are fractions of the t, g, h rotamers and J_T and J_G are the respective, standard trans and gauche coupling constants. Analogous systems of equations with f_{ts}, f_{gs}, f_{hs} and f_{tsc}, f_{gsc}, f_{hsc} rotamer populations can be set.

The systems of equations can be solved to obtain the rotamer populations, provided that the J_T and J_G parameters are available. For ABX spin systems in amino acid-like molecules, the most widely agreed parameter values are $J_T = 13.6$ Hz and $J_G = 2.56$ Hz.[33] Ambiguity in the J_T and J_G values causes 5−10% and 20−30% uncertainty in the population of the major and minor rotamers, respectively.[26]

The ^{1}H NMR data of NAC are collected in Table 1. The calculated rotamer populations along with related, earlier parameters of cysteine are compiled in Table 2.

Of all the rotamers, the most predominant one is t in basic solutions, indicating that the strongest intramolecular positioning force is the anionic repulsion between the thiolate and carboxylate sites. Upon neutralization of the thiolate, rotamer h becomes the most populous one. Rotamer h contains the three bulky groups in gauche position, which is, in cases of several amino acids, a crowded, hindered situation[19] and became therefore the origin of the designation h. Taking these aspects into account, the fact that h becomes highly populated shows the formation of a hydrogen-bonded system upon protonation of the anionic site(s). Rotamer g is the least populous one at every pH, since its occurrence is favored neither by anionic repulsions, nor by extended H-bond formation.

A striking resemblance can be observed between the rotamer populations of cysteine and *N*-acetylcysteine, at basic pH. The high similarity disappears upon protonation at any of the sites, especially for rotamers t and h. These phenomena also indicate that the most significant intramolecular conformational organizing force is the repulsion between the groups of high electron density. When any of these groups protonate, the differences between the *N*-acetyl and amino (ammonium) sites play relatively enhanced role, resulting

Table 2. Rotamer Populations of *N*-Acetylcysteine in Various States of Protonation, as Compared to Related Data of Cysteine[a]

N-acetylcysteine				cysteine			
	rotamer population, %				rotamer population, %		
protonation state	t	g	h	protonation state	t	g	h
S^-, NAc, COO^-	64	10	26	S^-, NH_2, COO^-	63	9	28
				S^-, NH_3^+, COO^-	52	15	33
SH, NAc, COO^-	37	14	49	SH, NH_2, COO^-	29	15	55
				SH, NH_3^+, COO^-	29	14	57
SH, NAc, COOH	38	18	44	SH, NH_3^+, COOH	28	16	56

[a] Latters are from ref 27.

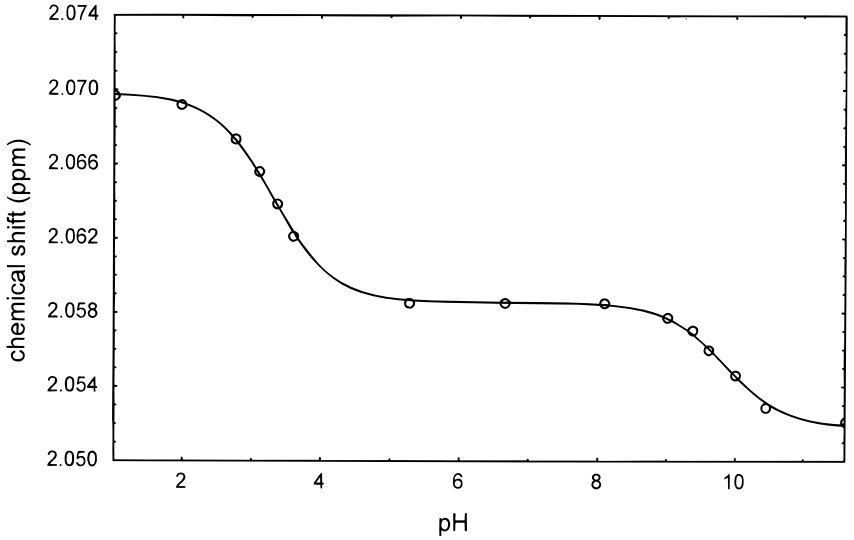

Figure 2. ^{1}H NMR chemical shifts of the *N*-acetyl-L-cysteine methyl protons as a function of pH.

in increased dissimilarities between the cysteine and *N*-acetylcysteine rotamer populations.

Rotamer h is always more populous in cysteine than in *N*-acetylcysteine, which is due to either the $-NH_2$ ($-NH_3^+$) < $-N-COCH_3$ relation in size or the NH_2 ($-NH_3^+$) > $-N-COCH_3$ relation in hydrogen-bonding capacity. In general, no real similarity can be found between rotamer populations of the two compounds when the amino site is cationic in cysteine.

Bulk Basicities. The most basic, dianionic form of *N*-acetylcysteine, NAC^{2-}, protonates in two steps:

$$NAC^{2-} + H^+ \rightleftharpoons NACH^- \quad (4)$$

$$NACH^- + H^+ \rightleftharpoons NACH_2 \quad (5)$$

The related, stepwise, macroscopic protonation constants, K_1 and K_2 can be expressed in terms of macrospecies concentrations:

$$K_1 = \frac{[NACH^-]}{[NAC^{2-}][H^+]} \quad (6)$$

$$K_2 = \frac{[NACH_2]}{[NACH^-][H^+]} \quad (7)$$

The observed ^{1}H NMR chemical shifts (δ_{obs}) depend on pH (Figure 2) and are related to the mole fractions and specific chemical shifts of the protonation forms, as follows:

$$\delta_{obs} = \delta_{NAC^{2-}}\alpha_{NAC^{2-}} + \delta_{NACH^-}\alpha_{NACH^-} + \delta_{NACH_2}\alpha_{NACH_2} \quad (8)$$

where

$$\alpha_{NAC^{2-}} = \frac{[NAC^{2-}]}{[NAC^{2-}] + [NACH^-] + [NACH_2]} = \frac{1}{1 + K_1[H^+] + K_1K_2[H^+]^2} \quad (9)$$

$$\alpha_{NACH^-} = \frac{[NACH^-]}{[NAC^{2-}] + [NACH^-] + [NACH_2]} = \frac{K_1[H^+]}{1 + K_1[H^+] + K_1K_2[H^+]^2} \quad (10)$$

$$\alpha_{NACH_2} = \frac{[NACH_2]}{[NAC^{2-}] + [NACH^-] + [NACH_2]} = \frac{K_1K_2[H^+]^2}{1 + K_1[H^+] + K_1K_2[H^+]^2} \quad (11)$$

Due to the well-separated stepwise protonation fashion of NAC, $\delta_{obs_{pH>12}} = \delta_{NAC^{2-}}$, $\delta_{obs_{8>pH>6}} = \delta_{NACH^-}$, and $\delta_{obs_{pH<1}} = \delta_{NACH_2}$. The K_1 and K_2 protonation macroconstants could be determined from all the four carbon-bound proton chemical shift series of the ^{1}H NMR spectra. The constants obtained so are $\log K_1 = 9.85 \pm 0.03$ and $\log K_2 = 3.31 \pm 0.02$. The corresponding values published by Inczédy[17] and Snyder[23] are very close to these, whereas other related data show moderate[22] or poor[24] agreement.

The thiolate protonation constant of NAC is interpreted in comparison with the analogous cysteine thiolate data.[27]

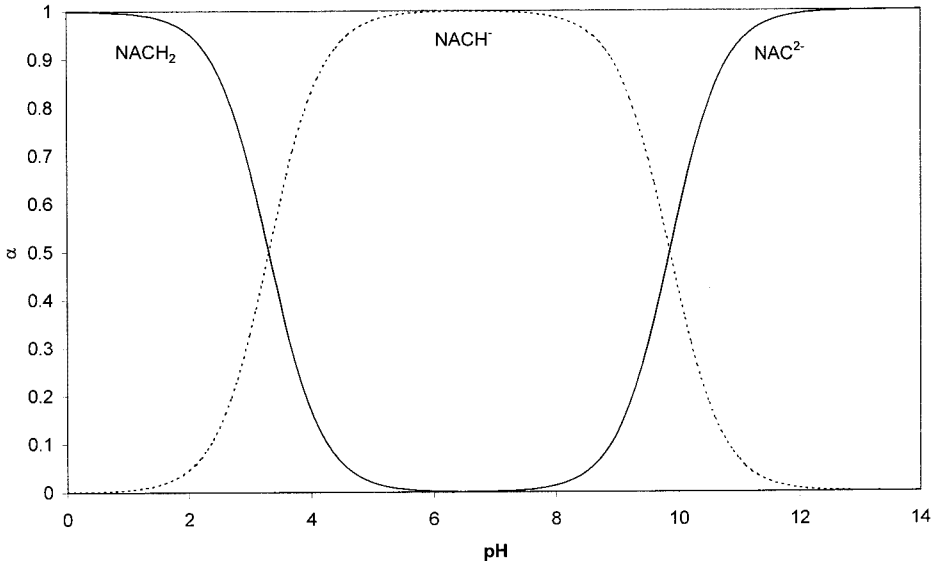

Figure 3. Distribution of NAC^{2-}, $NACH^{-}$, and $NACH_2$, the various protonation forms of *N*-acetyl-L-cysteine, as a function of pH.

The cysteine thiolate basicity is modulated by two pH-dependent intramolecular factors: (i) the protonation state of the adjacent amino site, which is the major modulating factor, and (ii) the state of rotamers, which produces minor effects.

Due to the strong electron-withdrawing effect upon protonation at the amino-site, two significantly different thiolate basicities belong to cysteine: $\log k^{S} = 10.00$ and $\log k^{S}_{N} = 8.45$. Former and latter represent the thiolate basicity when the side-chain amino-site is neutral and cationic ($-NH_2$ and $-NH_3^+$), respectively. The more basic form of thiolate in cysteine becomes predominant at pH > 10.15. Thus, the NAC thiolate basicity is close to the most basic thiolate stage of cysteine. An important difference is, however, that the $\log K_1 = 9.85$ value characterizes the NAC thiolate site throughout the range pH > 3.3. A thorough analysis of thiolate and amino−thiolate physicochemical properties in terms of microscopic basicity, microscopic redox potential, and effective reducing capacity will be published soon.[34]

The bulk carboxylate constant of NAC ($\log K_2 = 3.31$) is an expected value, characteristic of peptide C-terminal basicities, and is in accordance with the *N*-acetyl, amide-like, and $-CH_2-SH$ neighborhood of the carboxylate site.

The pH-dependent distribution of NAC macrospecies can be seen in Figure 3.

Evaluation and Interpretation of the Rotamer-Specific Basicities. As shown in Figure 1, the bulk species are composite ones of rotamers (conformers):

$$[NAC^{2-}] = [t] + [g] + [h] \tag{12}$$

$$[NACH^{-}] = [t_S] + [g_S] + [h_S] \tag{13}$$

$$[NaCH_2] = [t_{SC}] + [g_{SC}] + [h_{SC}] \tag{14}$$

Definition and evaluation of the rotamer-specific parameters is exemplified below by rotamer g:

$$g + H^+ \rightleftharpoons g_S \tag{15}$$

$$k_{1g} = \frac{[g_S]}{[g][H^+]} \tag{16}$$

where k_{1g}, quantitates the g-rotamer-specific thiolate basicity of NAC. Charges and protons (if any) on g, g_S, g_{SC}, t, t_S, etc. are omitted. Rather, the protonated thiolate or carboxylate sites are indicated in subscript.

The fraction of rotamer g in the pool of NAC^{2-} rotamers can be formulated as follows:

$$f_g = \frac{[g]}{[t] + [g] + [h]} = \frac{[g]}{[NAC^{2-}]} \tag{17}$$

The analogous expression for g_S, the thiolate-protonated g rotamer, is given below:

$$f_{g_S} = \frac{[g_S]}{[t_S] + [g_S] + [h_S]} = \frac{[g_S]}{[NACH^{-}]} \tag{18}$$

Expressing [g] and [g_S] from eqs 17 and 18 and introducing them into eq 16 yields

$$k_{1g} = \frac{f_{g_S}[NACH^{-}]}{f_g[NAC^{2-}][H^+]} = \frac{f_{g_S}}{f_g}K_1 \tag{19}$$

Thus, in general, the rotamer-definite constant is a structure-specified version of the bulk constant, modulated by the appropriate rotamer mole fractions.

The previously unreported relationship between the bulk and rotamer-specific basicities can be deduced as follows:

$$K_1 = \frac{[NACH^{-}]}{[NAC^{2-}][H^+]} = \frac{[t_S] + [g_S] + [h_S]}{([t] + [g] + [h])[H^+]} \tag{20}$$

Expressing [t_S], [g_S], and [h_S], the monoprotonated rotamer concentrations from the appropriate rotamer-specific constants, and rearranging yields

$$K_1 = \frac{k_{1t}[t][H^+] + k_{1g}[g][H^+] + k_{1h}[h][H^+]}{([t] + [g] + [h])[H^+]} \tag{21}$$

Table 3. Bulk and Rotamer-Specific Protonation Constants of *N*-Acetylcysteine

thiolate constants		carboxylate constants	
sign	value	sign	value
$\log K_1$	9.85	$\log K_2$	3.31
$\log k_{1t}$	9.61	$\log k_{2t}$	3.32
$\log k_{1g}$	9.97	$\log k_{2g}$	3.42
$\log k_{1h}$	10.12	$\log k_{2h}$	3.26

Introducing equations like (17) and simplifying yields

$$K_1 = k_{1t} f_t + k_{1g} f_g + k_{1h} f_h \qquad (22)$$

Equation 22 shows that the bulk protonation constant is a weighted sum of the rotamer-specific ones, where mole fractions of the unprotonated forms are the weighting factors.

Since rotamer-specific basicities are derived values of two parameters, their ambiguity necessarily exceeds that of the bulk constants. Conclusions will therefore be drawn from differences above the estimated 0.1 log *k* unit ambiguity only.

Table 3 contains all the rotamer-specific thiolate and carboxylate basicities of NAC. The thiolate constants show significant conformer dependence, whereas much smaller differences can be seen in the carboxylate basicities. These phenomena are consequences of the factors that influence the rotamer-specific constants. Namely, the rotamer-specific constants differ only from the bulk constant on the condition that protonation modifies the population of the rotamer in question (see eq 19). The largest thiolate basicity belongs to rotamer h, where both the carboxylate and *N*-acetyl moieties are in the gauche position and their high electron density supports the thiolate protonation. The significantly supporting group is the carboxylate, as shown by the $k_{1g} > k_{1t}$ relation. In fact, the trans carboxylate−thiolate position results in the lowest thiolate basicity (k_{1t}) of NAC.

The distribution of those species that are defined by both of their protonation and rotameric status can be obtained as the product of two appropriate fractions. To illustrate, α_{t_S}, the relative population of the monoprotonated *t* rotamer, is the product of α_{NACH^-}, a pH-dependent quantity (eq 10), and f_{t_S}, a pH-independent, rotamer-specific quantity:

$$\alpha_{t_S} = \alpha_{NACH^-} f_{t_S} \qquad (23)$$

The fraction of all the nine doubly specific species can be obtained analogously. Their distribution as a function of pH can be seen in Figure 4.

Oxidation−Reduction Properties. The observation that thiolate basicities and half-cell potentials of thiol−disulfide redox pairs are in correlation[31] makes the thiolate basicity values important not only in acid−base chemistry but also in oxidation−reduction chemistry. Thiolate basicities (log *K*) and the corresponding, pH-independent half-cell potentials ($\Delta E^{\circ\prime}$) have been determined in D_2O solutions for 10 thiol compounds, relative to the glutathione GSH/GSSG system. Thus, taking the $\Delta E^{\circ\prime}_{GSH/GSSG} = 0$ mV value for the reference compound, a total of 11 log K − $\Delta E^{\circ\prime}$ data pairs were applied to form a linear regression relationship, which resulted in the $\Delta E^{\circ\prime} = 63.89 \log K - 604.4$ equation, with the correlation coefficient $r = 0.966$. The correlation is close ($r = 0.977$) in the region of high thiolate basicities ($\log K > 8.5$). The transfer of log *K* data from 90% H_2O−10% D_2O to 100% D_2O medium can be done by adding 0.6 log *K* units,[26] in accordance with IUPAC recommendations.[35] The resulting, relative half-cell potential thus obtained for the NAC thiol/disulfide pair is +63 mV.

The relative half-cell potentials of small biomolecule thiol/disulfide systems span from −73 to +84 mV, being $\Delta E^{\circ\prime}_{GSH/GSSG} = 0$, in the center. Thus, NAC is certainly a strong reducing agent, especially among natural compounds and their close derivatives. Other compounds with similar half-cell potentials are captopril (+69 mV), 3-mercapto-propionic acid (+84 mV), and the $-NH_2$ microform of cysteine (+83 mV).

This protonation isomer of cysteine, however, becomes predominant above pH 10.15 only, a nonexisting medium in any compartment of the body. In real biological fluids, the characteristic cysteine half-cell potential is near −40 mV, a value that belongs to the $-NH_3^+$, $-S^-$, $-COO^-$ status of cysteine.

Another important comparison can be made with glutathione (GSH), the most abundant intracellular agent against oxidative stress. The thiolate basicity in GSH is approximately the same as that of typical thiolates in peptides and proteins, since the neighboring amino and carboxylate groups are involved in peptide bonds. Consequently, the strong disulfide-reducing and concomitant mucolytic character of glutathione cannot be expected.

These comparisons show that the adjacent *N*-acetyl and carboxylate groups (instead of the respective $-NH_3^+$ and −CONH− moieties) both stabilize the high electron density and the concomitant high basicity and strong reducing power of the thiolate site in NAC.

Since rotamer-specific basicities have been determined, rotamer-specific half-cell potentials could also be obtained, using the basicity-redox potential relationship. We believe, however, that the physical basis of the correlation is the electron density at the thiolate site. An inherent high electron density promotes both the release of an electron and the binding of a proton. Whether the rotational contribution of a gauche carboxylate to the proton binding at a thiolate site is related or not to the thiolate reducing power is not yet clear. We therefore have not elucidated rotamer-specific redox potentials.

The In Vivo Behavior of NAC at the Molecular Level. Many of the therapeutic, pharmacokinetic, and biochemical properties of NAC can be interpreted in the light of the above itemized physicochemical parameters.

As Figure 3 shows, $NACH_2$, the neutral species becomes predominant at pH ≤ 3.3, allowing membrane penetration from the gastric fluid by passive diffusion. This is in agreement with the observation[1,2] that orally administered NAC undergoes fast absorption and reaches peak plasma concentration in 90 min.[2] Figure 4 and Table 3 indicate that rotamer g_{SC} can well be assumed to be the best absorbing form from the stomach, since g_S typically receives the neutralizing proton at pH = 3.42, some 0.1−0.15 pH units higher than its rotameric counterparts. Furthermore, structural considerations

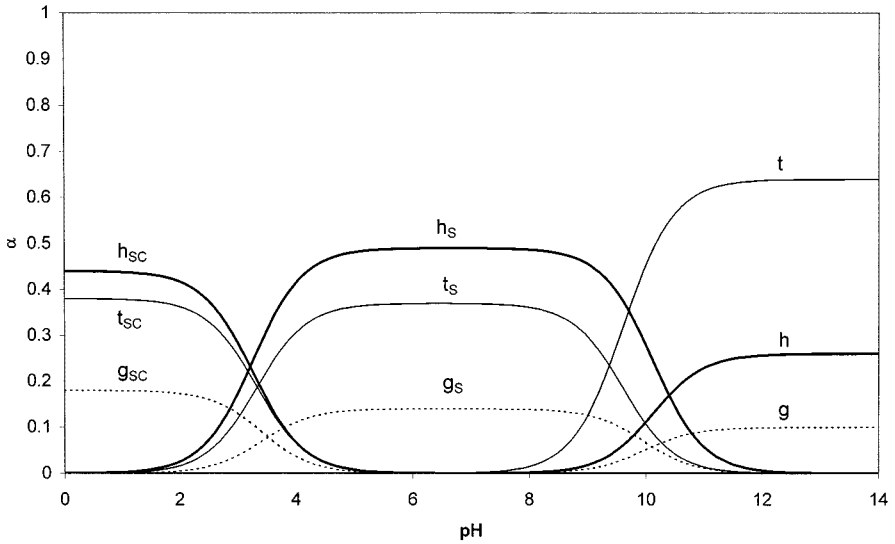

Figure 4. Distribution of the nine rotamer- and protonation-specific forms of *N*-acetyl-L-cysteine.

predict that the apolarity, hydrophobicity, lipophilicity order is $g_{SC} > t_{SC} > h_{SC}$.

Figure 3 also shows that $NACH_2$, the self-transporting form, constitutes as little as 10^{-3} % of the total NAC concentration at the pH of blood (7.4). In other words, the membrane-penetrating capacity of NAC from neutral medium is very poor either way. These data explain the observation that NAC produces hardly any radioprotective effects under in vivo conditions, if dosing is attempted by inhalation.[7] Also, once NAC entered the systemic circulation by the gastric (or possibly intravenous) route, it can only leave the blood vessels after decomposition, or by carrier-mediated active transport. No report appeared, however, on any active transport of NAC.

The lack of second penetration was experienced by respiratory studies, when no NAC could be detected in the bronchoalveolar lavage fluid after oral administration.[13]

Comparing, finally, its membrane-penetrating properties with those of cysteine, it can be presumed that cysteine is more mobile. While cysteine bears no gross charge at 2.1 < pH < 8.2, *N*-acetylcysteine is monoanionic at 3.3 < pH < 9.85 and dianionic at pH > 9.85. The higher mobility of cysteine can actually transfer a diminished extent of the reducing power of NAC through transmembrane processes.

Concerning the mucolytic activity, NAC has been reported to decrease the viscosity of bronchial secretions[13] and sputum[36] in direct, in vitro experiments.

In general, NAC has been stated to nonenzymatically detoxify reactive electrophiles and free radicals.[14]

These observations can be interpreted in terms of its +63 mV relative redox potential. This value enables NAC to lose an electron, which can be due to its participation either in a disulfide bond formation or in a spin-coupling process, as part of a radical-scavenging, detoxifying cascade.

The replacement of long peptide or protein chains by the small NAC moiety is a thermodynamically favored reaction on the basis of the pertinent, estimated 60 mV $\Delta E°$ difference and provides a reasonable explanation of the decreased viscosity.

The apparent contradiction that NAC treatment is accompanied by an increased glutathione level in the plasma[13] but that no NAC components can be detected in the plasma glutathione as proven by studies with isotope-labeled compounds[15] can also be resolved.

The higher disulfide-forming propensity of NAC can liberate the reduced form of glutathione (GSH) either from the homo- (GSSG) or the hetero-disulfide (GSSR) derivatives, in thiol−disulfide exchange reactions, shifted toward the formation of oxidized NAC. The same is true in reactions of NAC and cysteine-, homo-, and heterodisulfides, noting that the predominant form of cystine can be reduced more easily by NAC at neutral pH, due to the higher (∼100 mV) $\Delta E°'$ difference. The subsequent radical-scavenging and macromolecular disulfide-disrupting reactions can then be done by the liberated, reduced forms of glutathione and cysteine. These latter reactions take place with moderate thermodynamic ease, because of their limited reducing strength, but with enhanced efficacy, due to their increased concentration.

Experimental Section

N-Acetyl-L-cysteine, sodium deuterioxide, and deuterium chloride were obtained from Sigma Chemical Co., and deuterium oxide was from Merck. Bidistilled water was used for all experiments.

The 0.1 M *N*-acetylcysteine solutions were prepared in 10% D_2O and 90% H_2O mixtures; 2 M DCl/HCl and 2M NaOD/NaOH 10/90 solutions were used to set the pH.

The pH−potentiometric measurements were carried out with Radiometer PHM93 reference pH meter, equipped with Orion 9103BN semi-micro combination electrode. The pH−potentiometric system was standardized using aqueous pH = 4.005 and 9.180 buffer solutions. The pH values reported are pH meter readings.

Vicinal proton−proton coupling constants at pH = 0.98, 7.13, and 12.12 were obtained at 500.1 MHz, using a Bruker DRX-500 spectrometer. At these pH values the three distinct protonation states of NAC exist almost exclusively. ^{1}H NMR titrations were carried out in solutions of 15 different pH values, ranging from 0.5 to 13, at 200.1 MHz and 298 K with a Bruker AM 200 NMR spectrometer. A 90° pulse and a spectral range of 1200 Hz were used. The free induction decay was digitized into 32K data points. Typically 16 transients were coadded and a 15 s repetition time was used.

Chemical shifts were measured relative to internal *tert*-butyl alcohol (1.236 ppm). Coupling constants were evaluated from spectra recorded at 500 MHz by non-first-order spectral analysis.[37]

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