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The use of amino compounds for binding 2,4,6-trinitrotoluene in water

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"Capsule": *Addition of aniline and an amino acid-like cysteine to water decreased free TNT.*

Abstract

Sites polluted with 2,4,6-trinitrotoluene (TNT) constitute a worldwide problem. In this work, chemical reactions for binding TNT to amino-compounds are proposed as an initial step for developing new remediation techniques to clean-up groundwater and soils contaminated with TNT. Indeed, addition of aniline and an amino acid-like cysteine caused a decrease in free TNT of 86% and 68–100%, respectively. Using ^{13}C-NMR spectroscopy, it was shown that TNT chemically forms a Meisenheimer complex with cysteine and aniline in 1/1 (by vol.) H_2O/d_6-acetone.

Keywords: TNT; Amino acid; Meisenheimer complex

1. Introduction

In the past decades, nitroaromatics have been widely disposed to soil and groundwater as dyes, pesticides, and explosives (Hartter, 1985). Among nitroaromatic compounds, 2,4,6-trinitrotoluene (TNT) has been known as one of the widespread, mutagenic, and recalcitrant pollutants (Won et al., 1976; Walker and Kaplan, 1992; Rieger and Knackmuss, 1995). Many years after the production activities were stopped, TNT concentrations of 80 mg/l and 500 μg/l have been found in process and groundwaters, respectively (Hao et al., 1993; Price et al., 1997). These sites need to be remediated as the potential hazard of TNT to human health as well as its toxicity for higher and lower organisms have been demonstrated. Several treatment techniques for TNT-contaminated water are known such as biotreatment, carbon adsorption, incineration, wet air oxidation and chemical transformation (Spain, 1995; Hundal et al., 1997; Held et al., 1997). Binding processes constitute a potential to initiate soil clean-up (Verstraete and Devliegher, 1997). In this study, a chemically based reaction for binding TNT to amino-compounds, such as cysteine and aniline, was demonstrated for the first time as a novel approach for designing such remediation processes. ^{13}C-NMR spectroscopy was used to identify the Meisenheimer complex formed between TNT and cysteine or aniline. Hydride–Meisenheimer complexes have been clearly identified as playing a key role in the biodegradation of nitroaromatic compounds (Spain, 1995; Haidour and Ramos, 1996; Rieger et al., 1999).

2. Materials and methods

2.1. Treatment of water polluted with TNT by addition of several amino compounds

A mixture of 1/1 (by vol.) H_2O/d_6-acetone with a concentration of 0.2 mM TNT (Sigma Aldrich, Belgium) was used for initial tests. The remaining amount of TNT was measured 2 h after addition of 5.5 mM cysteine (VEL, Belgium), 5.5 mM Fe^{2+}/Fe^{3+} (VEL, Belgium) and a combination of both.

For subsequent tests, several L-amino acids (arginine, methionine, serine, tyrosine, lysine, glycine, cysteine;

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VEL, Belgium) were added at a final concentration of 0.9 mM. The solution contained 0.3 mM TNT.

To investigate the role of the amino group, equimolar quantities of aniline, allylthiourea, diethylamine and diphenylamine were added to a solution containing 0.4 mM TNT. The residual amount of TNT was determined after one day–night cycle.

On a solution of 0.3 mM TNT, the concentration dependency for cysteine and aniline was measured for final amino-compound concentrations of 0.0, 0.3, 0.9, and 3.0 mM. One-half of the solution was kept in the dark and the other half was exposed to 10 day–night cycles.

2.2. Photospectrometry

TNT was quantified by a photospectrometric method described by Jenkins (1990). Fifteen millilitres of acetone was added to 10 ml of the water sample. Then, one drop of 10% tetrabutylammonium buffer solution was added and the red coloration measured after 15 min at 550 nm.

2.3. NMR spectroscopy

Due to the insensitivity of this technique, the samples of 1/1 (by vol.) H_2O/d_6-acetone contained 4 mM TNT and 12 mM cysteine, except for the equimolar solution that contains 4 mM TNT and 4 mM cysteine. The same equimolar concentrations were used for the aniline/TNT solution (in acetone) and for TNT dissolved in acetone and in 9/1 (by vol.) acetonitrile/d_3-acetonitrile. The chemical shift is referenced relative to the methyl of acetone (at 29.2 ppm for ^{13}C). Small amounts of chromium acetylacetonate (Ventron Alfaproducts, USA) were added to reduce the relaxation times in order to observe all quaternary carbon resonance signals.

The 1D proton decoupled ^{13}C-NMR spectra were recorded at a ^{13}C frequency of 90.6 MHz and at room temperature. The spectral width was 21.7 kHz (70,000 scans) and no relaxation delay was used. The spectral resolution of the 32 K spectra was 1.3 Hz/pt. Prior to Fourier transformation, the data were multiplied with an exponential window function (LB 3.0 Hz) to increase the sensitivity.

3. Results and discussion

3.1. Treatment of water polluted with TNT by addition of several amino-compounds

VanBeelen and Burris (1995) described a reduction of free TNT in the presence of Fe^{2+} and cysteine provided 1 h of incubation. This experiment was repeated and the results showed that iron is not required to decrease the amount of free TNT when cysteine was added. Indeed the addition of cysteine eliminated 96% of free TNT while the addition of Fe(II) or Fe(III) to a TNT solution caused 91–94% disappearance of TNT. Simultaneous treatment with cysteine and iron had no additional effect on the decrease of free TNT compared to the treatment with cysteine alone.

In order to gain more insights on the reaction mechanism, several amino acids were tested at a concentration of 0.9 mM. After one day–night cycle of incubation, 68–100% of free TNT disappeared from the solution (Table 1). Thus, it seems that different types of amino acids could decrease the amount of free TNT in water. To identify which of the two functional groups of the amino acid (the amino or acid group) is involved in reducing free TNT, equimolar quantities of several amines were added to the TNT solution. Among four amino-compounds tested, only aniline caused a significant decrease of TNT as 86% of free TNT was eliminated (Table 1). On this basis it can be concluded that the amino group is the functional group that binds TNT. However, not only is this functional group important but also the overall structure of the compound. In the case of diphenylamine, no TNT disappearance was observed. An explanation could be that the formation of a Meisenheimer complex (see below) is probably hampered due to sterical hindrance of the two aromatic rings.

The amount of amino-compound necessary to get 100% TNT disappearance has been investigated and Table 2 shows that there is a minimal concentration of 3 mM cysteine or 0.3 mM aniline needed to totally bind 0.3 mM TNT. These results suggested that aniline binds TNT better than cysteine.

When a TNT solution was standing for some days in daylight, a red coloration due to a photochemical reaction (Schmelling et al., 1998) was observed. The influence of light on a TNT solution in the presence of cysteine or

Table 1
Effect of addition of amino acids (0.9 mM) and amines (0.9 mM) on the disappearance of free 2,4,6-trinitrotoluene (TNT) after 1 day of incubation

Solution	TNT decrease (%)
TNT (0.3 mM)	0
TNT (0.3 mM) + arginine	68
TNT (0.3 mM) + methionine	84
TNT (0.3 mM) + serine	99
TNT (0.3 mM) + tyrosine	99
TNT (0.3 mM) + lysine	100
TNT (0.3 mM) + glycine	99
TNT (0.3 mM) + cysteine	98
TNT (0.4 mM) + aniline	86
TNT (0.4 mM) + allylthiourea	0
TNT (0.4 mM) + diethylamine	0
TNT (0.4 mM) + diphenylamine	0

Table 2
Decrease in free 2,4,6-trinitrotoluene (TNT) (0.3 mM) for different concentrations of cysteine and aniline, incubated in daylight and in darkness, after one and 10 day–night cycles

Solution		Decrease (%)	
		After 1 day	After 10 days
TNT	Daylight	12	45
	Darkness	0	–
TNT + 0.3 mM cysteine	Daylight	95	67
	Darkness	81	2.2
TNT + 0.9 mM cysteine	Daylight	100	82
	Darkness	100	33
TNT + 3 mM cysteine	Daylight	100	100
	Darkness	100	100
TNT + 0.3 mM aniline	Daylight	98	100
	Darkness	100	100
TNT + 0.9 mM aniline	Daylight	100	100
	Darkness	100	100
TNT + 3 mM aniline	Daylight	100	100
	Darkness	100	100

aniline was tested. The results showed that the influence of light on the binding reaction was only detected when the concentrations of cysteine and aniline were lower than the minimal amount needed for 100% binding (Table 2).

3.2. Determination of the Meisenheimer complex between TNT and cysteine or aniline by ^{13}C-NMR spectroscopy

Anionic σ-adducts, also termed Meisenheimer complexes, arise from covalent bond formation between nucleophiles and electron-deficient nitroaromatic compounds (Buncel et al., 1995). Meisenheimer complexes were first identified by ^{13}C-NMR by Olah and Mayr (1976). The ^{13}C chemical shifts of the carbons in the ring are the best parameters to locate the exact binding position, because there is a linear relation between the ^{13}C chemical shift and the charge distribution in the ring (Olah and Mayr, 1976).

A 1D ^{13}C spectrum of a TNT solution in acetonitrile was used to assign all the chemical shifts of TNT (Table 3). In the 1D ^{13}C spectrum of the TNT sample in water/d_6-acetone (Fig. 1a), only the chemical shift of C_1 does not agree with the chemical shifts measured in the acetonitrile solution of TNT. This chemical shift has an upfield shift of 34.6 ppm which is consistent with the fact that acetone also forms a Meisenheimer complex with TNT (Foster and Fyfe, 1966). This was confirmed by an upfield shift of 3.0 ppm of the methyl carbon atoms of acetone, and the fact that the TNT solution also has a red colour.

In the 1D ^{13}C spectrum of a mixture of 4 mM TNT and 12 mM cysteine, two sets of signals appeared for cysteine, one from uncomplexed (free) and the other from complexed cysteine (Table 3). Both forms could be distinguished based on the difference in the intensity of their signals. The ^{13}C signals of cysteine are shifted upfield by approximately 10 ppm after complexation with TNT. All carbon signals of cysteine are doubled, with the exception of the carbonyl carbon. Comparison of the ^{13}C resonance signals of TNT before and after addition of cysteine (Table 3) revealed that by adding cysteine the signal of C_1 moved from 98.6 to 72.8 ppm (Fig. 1). After incubation of the equimolar sample during a day–night cycle, the signal of C_1 at 72.8 ppm moved to aliphatic region ($\delta = 48.5$ ppm) which is characteristic for a sp^3 carbon (Fig. 1).

Analysis of the 1D ^{13}C-NMR spectra of the equimolar aniline/TNT mixture (Table 3) showed that the chemical shift of the carbon atoms closest to the amino group are shifted upfield. This could be due to the proximity of the aromatic ring of TNT. The change in hybridisation of the C_1 atom of TNT after complex formation results in an upfield shift of 85.7 ppm of the chemical shift of this atom (Table 3).

Using NMR spectroscopy, it is thus shown that TNT forms a Meisenheimer complex with cysteine and aniline in 1/1 (by vol.) H_2O/d_6-acetone (Scheme 1). The chemical shift of C_1 of TNT is by far the largest upfield shift (from 50 up to 90 ppm) since the hybridisation of

Table 3
^{13}C chemical shifts (in ppm) of 2,4,6-trinitrotoluene (TNT), cysteine and aniline for different samples at room temperature

	Chemical shift (ppm)					
	TNT[a]	TNT[b]	TNT/Cys[c]	TNT/Cys[d]	Aniline[e]	TNT/aniline[f]
TNT						
C_1	134.5	98.6	72.8	48.5	–	48.8
$C_{2,6}$	151.9	150.9	150.9	150.9	–	151.6
$C_{3,5}$	123.1	122.5	122.5	122.5	–	122.4
C_4	146.6	145.3	145.3	145.3	–	146.3
C_7	15.4	14.9	14.9	14.9	–	14.9
Cys						
CO	–	–	171.1	171.1	–	–
C^{α}_{free}	–	–	64.3	–	–	–
$C^{\alpha}_{complex}$	–	–	55.9	55.9	–	–
C^{β}_{free}	–	–	33.1	–	–	–
$C^{\beta}_{complex}$	–	–	24.9	24.9	–	–
Aniline						
C_1	–	–	–	–	147.9	125.9
$C_{2,6}$	–	–	–	–	116.3	94.1
$C_{3,5}$	–	–	–	–	130.0	129.7
C_4	–	–	–	–	119.2	120.3

[a] 4 mM TNT solution in 9/1 (by vol.) acetontrile/d_3-acetonitrile.
[b] 4 mM TNT solution in 1/1 (by vol.) H_2O/d_6-acetone.
[c] 4 mM TNT and 12 mM cysteine in 1/1 (by vol.) H_2O/d_6-acetone.
[d] 4 mM TNT and 4 mM cysteine in 1/1 (by vol.) H_2O/d_6-acetone after one day–night cycle incubation.
[e] Previously reported chemical shift values for aniline (11).
[f] 4 mM TNT and 4 mM aniline in 1/1 (by vol.) H_2O/d_6-acetone.

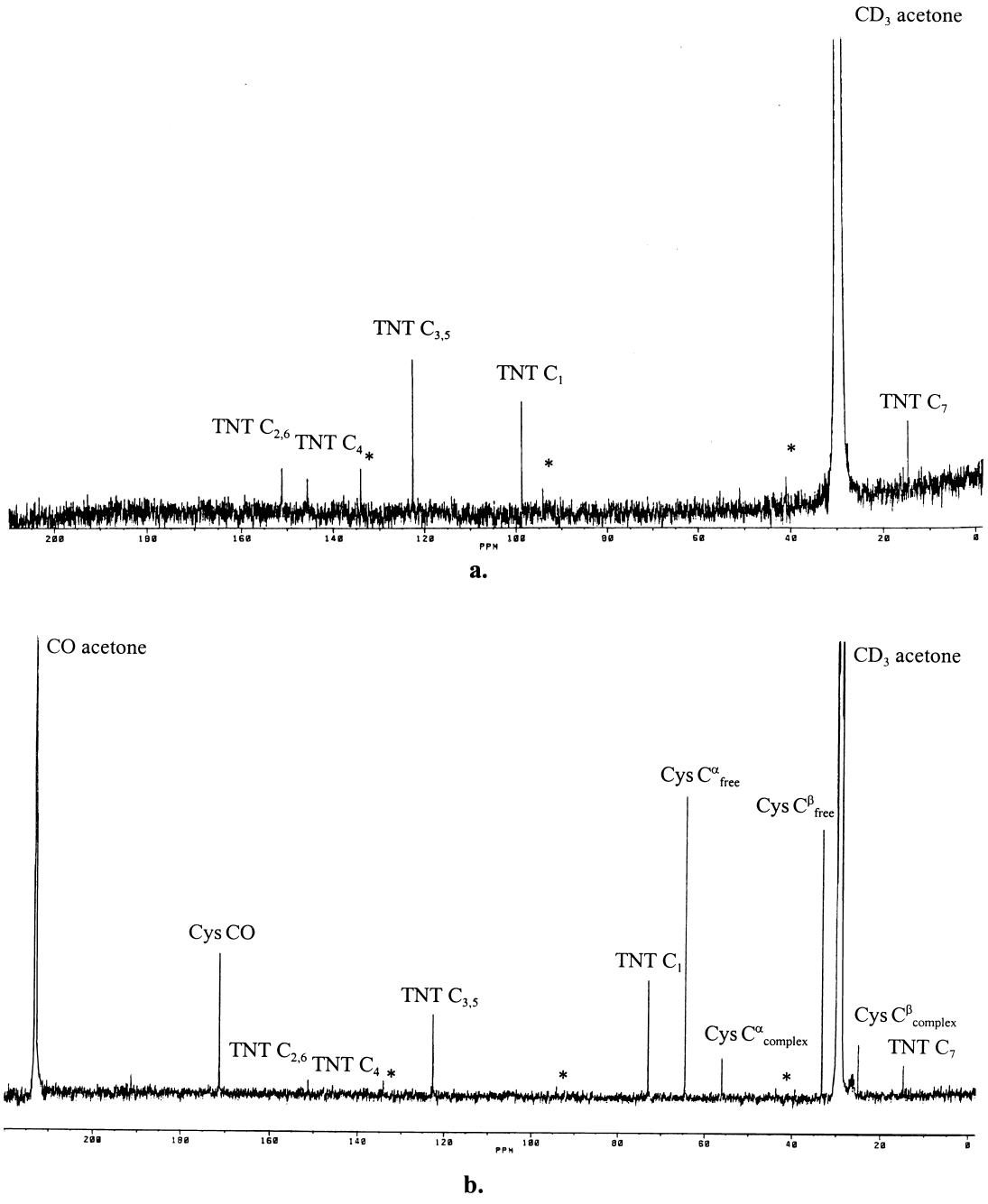
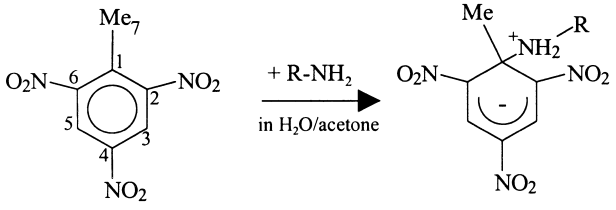


Fig. 1. ^{13}C-NMR spectrum of 2,4,6-trinitrotoluene (TNT) (4 mM) (a) without and (b) with cysteine (12 mM) in 1/1 (by vol.) H_2O/d_6-acetone at room temperature. The resonance signals of chromium acetylacetonate are indicated by an asterisk.

this atom changes from sp^2 to sp^3 during formation of the Meisenheimer complex (Buncel et al., 1995). If the covalent bond is formed with C_3 of TNT or if the anilide from the aniline is formed, the observed chemical shifts will be totally different. Thus, this complex is preferentially formed on the C_1 position but due to the presence of some low abundance impurities it cannot be ruled out that a Meisenheimer complex is formed at other positions. Alternatively, these impurities could be a result of disulphide bond formation by the cysteine or solvent impurities. Under biological conditions it has been shown



Scheme 1. Schematic representation of the formation of a Meisenheimer complex between 2,4,6-trinitrotoluene (TNT) and amino compounds (R-NH_2).

that some bacteria can reduce the aromatic ring of dinitro- and trinitro-compounds by the addition of a hydride ion to form a hydride–Meisenheimer complex, which subsequently re-aromatizes with the elimination of nitrite (Spain, 1995; Haidour and Ramos, 1996). Recently, Rieger et al. (1999) reported the evidence for the formation of a hydride–Meisenheimer complex of picric acid (2,4,6-trinitrophenol) and its protonated form under physiological conditions using *Rhodococcus erythropolis* HLPM-1. It was also proved that these complexes are key intermediates of denitration and productive microbial degradation of picric acid (Rieger et al., 1999).

4. Conclusions

This work showed that in a H_2O/d_6-acetone mixture, TNT forms a Meisenheimer complex with cysteine and aniline. When an overdose of cysteine is present, the equilibrium moves entirely to the Meisenheimer complex. After exposure to daylight, the formation of the complex is more enhanced. The addition of cysteine, aniline or crude protein extracts to water and soils polluted with TNT or any other nitroaromatic compound could be used as a simple and rapid process for binding the pollutant and initiating further biological remediation (Verstraete and Devlieghere, 1997).

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