REVIEW ARTICLE

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The chemistry of soil organic nitrogen: a review

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Abstract 1. From the data presented herein it is possible to deduce the following distribution of total N in humic substances and soils: proteinaceous materials (proteins, peptides, and amino acids) – ca. 40%; amino sugars – 5–6%; heterocyclic N compounds (including purines and pyrimidines) – ca. 35%; NH_3 –19%; approximately 1/4 of the NH_3 is fixed NH_4^+ . Thus, proteinaceous materials and heterocyclics appear to be major soil N components.

2. Natural ^{15}N abundance levels in soils and humic materials are so low that direct analysis by ^{15}N NMR is very difficult or impossible. To overcome this difficulty, the soil or humic material is incubated with ^{15}N -enriched fertilizer. Even incubation in the laboratory for up to 630 days does not produce the same types of ^{15}N compounds that are formed in soils and humic materials over hundreds or thousands of years. For example, very few ^{15}N -labelled heterocyclics are detected by ^{15}N NMR. Does this mean that heterocyclics are not present? Or are the heterocyclics that are present not labelled under these experimental conditions and therefore not detected by the ^{15}N NMR spectrometer? Another possibility is that a large number of N heterocyclics occur in soils, but each type occurs in very low concentrations. Until the sensitivity is improved, ^{15}N NMR will not provide results that can be compared with data obtained from the same soil and humic material samples by chemical methods and mass spectroscopy.

3. What is most important with respect to agricultural is that all major N forms in soils are available to organisms and are sources of NH_3 or NH_4^+ for plant roots and microbes. Naturally, some of the NH_3 will enter the N cycle.

4. From chemical and pyrolysis-mass spectrometric analyses it appears that N heterocyclics are significant components of the SOM, rather than degradation products of

other molecules due to pyrolysis. The arguments in favor of N heterocyclics as genuine SOM components are the following:

a) Some N-heterocyclics originate from biological precursors of SOM, such as proteinaceous materials, carbohydrates, chlorophyll, nucleic acids, and alkaloids, which enter the soil system as plant residues or remains of animals.

b) In aquatic humic substances and dissolved organic matter (DOM) at considerably lower pyrolysis temperatures (200 to 300°C), free and substituted N-heterocyclics such as pyrroles, pyrrolidines, pyridines, pyranes, and pyrazoles, have been identified by analytical pyrolysis (Schulten et al 1997b).

c) Their presence in humic substances and soils was also detected without pyrolysis by gel chromatography – GC/MS after reductive acetylation (Schnitzer and Spiteller 1986), by X-ray photoelectron spectroscopy (Patience et al. 1992), and also by spectroscopic, chromatographic, chemical, and isotopic methods (Ikan et al. 1992).

5. While we can see light at the end of the tunnel as far as soil-N is concerned, further research is needed to identify additional N-containing compounds such as N-heterocyclics, to determine whether these are present in the soil or humic materials in the form in which they were identified or whether they originate from more complex structures. If the latter is correct, then we need to isolate these complex N-molecules and attempt to identify them.

Key words Analytical pyrolysis · Humic substances · Heterocyclic nitrogen · ^{15}N NMR · Mass spectrometry · Soil organic matter · Model structure · Unidentified nitrogen

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Introduction

Except possibly for small amounts of geogenic (mineralogically-fixed) nitrogen (N), N is the only essential plant nutrient that is not released by the weathering of minerals

in soils. It is required in relatively large concentrations by most agricultural crops, but only trace quantities are available in mineral forms in the soil at any one time. The source of soil nitrogen is the atmosphere, where dinitrogen (N_2) is the predominant gas (79%). Only a few microorganisms have the ability to use molecular N_2 ; all remaining living organisms require combined N for carrying out their life activities. Increases in the level of soil N occur through the fixation of N_2 by some microorganisms and from the return of ammonia and nitrate in rain water; losses are due to harvesting of crops, leaching, and volatilization. Atmospheric ammonia originates from volatilization from soil surfaces, lightening, fossil fuel combustion, and natural fires. N is essential for crop production as it is an important constituent of proteins, nucleic acids, porphyrins, and alkaloids. Soil organic matter (SOM), especially humic substances, acts as a storehouse and supplier of N for plant roots and microorganisms; almost 95% of total soil N is closely associated with SOM.

While a considerable amount of research has been done on soil N over the years, most of this work has been limited to the qualitative and quantitative determination of proteinaceous materials, amino acids, and inorganic N compounds. Recent reviews on soil N summarize the results of qualitative and quantitative determinations on proteinaceous materials, amino acids, and other known organic N forms in soils (Kelley and Stevenson 1995), their mineralization and importance to plant nutrition (Mengel 1996). This means that about half the remaining sources of soil N are unidentified and poorly understood. Thus, there is a need for more research and information in this area.

The objectives of this review are to present an account of what we currently know, and do not know, about soil N. The first part of this review will deal with the distribution in soils of proteinaceous materials, amino sugars, and ammonia, while the second part will focus on more recent data on the identities and functions of heterocyclic N compounds, which also appear to play a significant role in supplying plant roots and microbes with N.

Important reactions of N in soils

Of special interest in the context of this discussion are the following four reactions, which involve N associated with SOM:

1. Nitrogen (N_2) in the atmosphere $\rightarrow$ organic N (nitrogen fixation)
2. Organic N $\rightarrow$ ammonia (mineralization or ammonification)
3. Ammonia $\rightarrow$ organic N (immobilization or assimilation)
4. Nitrate $\rightarrow$ organic N (nitrate assimilation or immobilization)

N fixation (reaction 1) involves the reduction of elemental N_2 to the -3 oxidation state in NH_3 . This biological process is catalyzed by nitrogenase, a large metalloenzyme.

The NH_3 produced is retained by N-fixing cells and reacts with glutamate to form glutamine. Newly fixed NH_3 is only rarely released by healthy N-fixing cells and must pass through an organic form before entering the N cycle (Smith 1982).

The mineralization of N (reaction 2) is carried out mainly by microorganisms. Through this process organically bound N is liberated as NH_3 . Whether N is mineralized or immobilized by microorganisms depends on the C/N ratio of the substrate compared to that of decomposer organisms. If the substrate has a low C/N ratio, N will be in excess and NH_3 will be liberated.

Immobilization (reaction 3) of N can occur through both biotic (Balabane and Balesdent 1996) and abiotic processes. NH_4^+ is efficiently immobilized by clay minerals in exchangeable and fixed forms. Exchangeable NH_4^+ is available for biological immobilization. Data by Rosswall (1982) show that in most soils 30–60% of the fixed NH_4^+ is also available for biological uptake. Addition to soils of a substrate with a high C/N ratio will bring about rapid microbial immobilization of NH_4^+ (Mengel 1996).

Nitrification refers to the oxidation of NH_3 to NO_2^- and/or NO_3^- , mainly by autotrophic nitrifying bacteria of the genera *Nitrosomonas* and *Nitrobacter*. Nitrification is a key process for determining the fate of N in soils. NO_2^- and NO_3^- are more mobile than NH_3 and therefore are more readily lost through leaching. NO_2^- can be reduced to N_2O (nitrous oxide) and N_2 by denitrifying bacteria. The main factor influencing the nitrification rate is the concentration of available NH_3 . The reduction of NO_3^- to NH_3 occurs in soils at low rates (Rosswall 1982). If it were possible to stimulate the reduction of NO_3^- to NH_3 and its incorporation into organic matter (reaction 4), large N losses resulting from leaching or denitrification could be prevented.

N distribution in soils

Sowden et al. (1977) determined the distribution of the major N compounds in samples taken from soils formed under widely different climatic and geological conditions on the earth's surface. The soil samples came from arctic, subarctic, cool temperate, subtropical, and tropical regions. All samples were hydrolyzed and analyzed by the same methods, which provided a type of uniformity which had not been attained before, and made it possible to gain new insights into the distribution of N in soils. Definitions of the different classes of N compounds as employed by Sowden et al. (1977) are listed in Table 1. While the total N content of the samples analyzed varied from 0.01% to 1.61%, the proportions of total N that could be hydrolyzed by hot 6 M HCl were quite similar (84.2% to 88.9%). Amino acid N ranged from 33.1% to 41.7%, amino sugar N from 4.5% to 7.4%, and ammonia from 18.0% to 32.0%. Some of the ammonia probably originated from amides, the decomposition of hydroxy- and other amino acids, amino sugars, the deamination of purines and pyrimidines, and the release of fixed NH_4^+ from clays. Amino

Table 1 Definitions used in this review

Hydrolyzable N = % of total N hydrolyzed by hot 6 M HCl in 24 h
Nonhydrolyzable N (NH-N) = 100% hydrolyzable N
Unidentified N (UN-N) = 100 (% amino acid N + amino sugar + NH ₃ -N)
Unidentified hydrolyzable N = UH-N
Protein N = % amino acid N + 10% amino acid N (to include amide N of asparagine and glutamine lost during 6 M HCl hydrolysis)
% Non-protein-N = 100% protein N

acid N and amino sugar N constituted greater proportions of the total N in soils from cooler regions. The reverse tended to be true for NH₃-N. Proportions of unidentified hydrolyzable N (UH-N) (16.5% to 17.8%) and those of nonhydrolyzable N (NH-N) (11.1% to 15.8%) were similar in all soils examined. Of special interest are the UH-N and NH-N fractions which constitute between 28% and 34% of the total soil N. Very little is known about the chemical composition of these fractions except that the N in these materials is not protein N, peptide N, amino acid N, nor amino sugar N or NH₃-N. In the second part of this review special attention will be given to the chemistry of these two fractions.

Estimates of non-protein N ranged from 55% for the tropical soils to 64% for the arctic soils, averaging 61% for all soils. Thus, about 60% of the total N in soils was

non-protein N or, conversely, 40% of the total soil N was protein N. In more recent work, acid hydrolysis was used to determine organic N forms in different soils and their particle-size fractions (Catroux and Schnitzer 1987; Christensen 1996), to observe transformations of labelled, inorganic N fertilizers (Sulce et al. 1996) and to investigate effects of manure applications (Sharpley and Smith 1995), cultivation of virgin soils (Stevenson 1986), and soil management in long-term agricultural experiments (Hersemann 1987; Leinweber and Schulten 1997). Results of these studies are compiled in Table 2. Sulce et al. (1996) and Sharpley and Smith (1995) observed relatively high proportions of nonhydrolyzable N, to a maximum of 47% of total N. Cultivation, manuring and other agricultural practices can alter the proportions of hydrolyzable and nonhydrolyzable N. In some studies, the proportion of nonhydrolyzable N was found to be relatively higher in unfertilized or intensively managed soils, whereas the application of farmyard manure led to an increased hydrolyzability of the organic N compounds present (Hersemann 1987; Leinweber and Schulten 1997). In contrast, Sharpley and Smith (1995) reported higher proportions of nonhydrolyzable N in manured compared to non-manured soils. Hence, the importance of this organic N fraction in agriculture is not completely clear, probably because of a lack of knowledge concerning its chemical identity and properties.

Since hot acid hydrolysis was required to release practically all of the amino acids and amino sugars from soils in the studies cited above, it is likely that the amino acids oc-

Table 2 Distribution of organic N forms in soils of different climatic zones, soil types, and from agricultural experiments in Belgium (B), Germany (D), The Netherlands (NL), United Kingdom (UK) and the United States of America (US). Standard deviations in square brackets

References	Climatic zones, soil units, experimental sites	NH ₃ -N	Amino N + UH-N	NH-N
Sulce et al. (1996)	Arenosol	19.2	43.3	37.5
	Cambisol	28.6	37.0	34.5
	Vertisol	27.4	43.1	29.5
	Vertisol	33.3	47.9	18.8
	Calcisol	25.8	57.5	16.7
	Fluvisol	26.8	51.2	22.0
	Fluvisol	29.3	50.2	20.5
Sharpley and Smith (1995)	Fluvisol	26.6	53.8	19.6
	Mollisols	24.5 [1.0]	44.2 [0.5]	31.2 [0.5]
	Ultisols	19.7 [8.4]	48.5 [7.2]	34.8 [5.8]
Stevenson (1986)	Alfisols	20.6 [8.7]	48.7 [8.0]	30.4 [10.7]
	Illinois (US)	16.6–16.7	49.4–52.5	20.2–20.3
	Iowa (US)	22.2–24.7	28.8–31.4	24.0–25.4
Hersemann (1987)	Nebraska (US)	19.8–24.5	42.8–51.6	19.3–20.8
	Bonn (D)	26.5–29.6	49.5–54.8	24.0–17.1
	Puch (D)	21.0–23.9	43.6–55.0	32.5–23.9
	Gembloux (B)	22.6–24.2	42.2–46.7	33.8–30.4
	No-Polder (NL)	23.7–25.4	44.2–46.0	31.2–24.3
	Barnfield (UK)	20.4–23.9	39.4–46.8	38.1–32.5
	Hoosfield (UK)	19.6–23.0	47.3–49.3	31.1–29.3
	Broadbalk (UK)	18.0–33.9	33.7–57.2	32.4–24.2
Leinweber and Schulten (1997)	Thyrow (D)	21.2–26.0	40.7–52.8	8.4–13.3
	Halle (D)	28.0–28.5	42.3–48.7	20.0–25.4
	Halle (D)	30.9–31.0	47.7–51.3	21.3–27.4
	Lauterbach (D)	26.1–29.2	50.7–54.1	12.6–17.2
	Lauchstädt (D)	28.4–29.9	47.6–50.3	21.2–21.3

cur in soils in the form of proteins and peptides closely associated with and protected by humic materials and inorganic soil constituents such as clay minerals and hydrous oxides of iron and aluminum. Similarly, amino sugars do not appear to exist as free compounds. Soil peptides were investigated after mild extraction by gel permeation chromatography and reverse-phase HPLC (Warman and Isnor 1991). The detected peptides had molecular weights ranging from 675 to 99370 daltons and contained 16 different amino acids. Their contribution to the total soil N varied for different soils and management practices.

To investigate whether hydrolysis with hot 6 M HCl hydrolyzed all of the proteinaceous materials in soils and humic substances, Griffith et al. (1976) hydrolyzed a number of soils and humic acids with hot 6 M HCl. Separate samples of the acid-treated residues were then hydrolyzed in sealed tubes with either 0.2 M Ba(OH)₂, or with 2.5 M NaOH under reflux. No significant differences were found between results obtained by the two types of hydrolysis. The data showed that hot 6 M HCl released almost all of the amino acids in the soils and humic acids in 24 h. Subsequent alkaline hydrolysis of the acid-treated residues freed only small additional amounts of NH₃-N (5% to 15%), which most likely originated from the hydrolyzable unidentified (HU-N) and unidentified nonhydrolyzable (NH-N) fractions.

Amino acids in soils

The amino acid composition of the soils investigated by Sowden et al. (1977) was remarkably similar, except for the following differences: (1) tropical soils rich in amorphous aluminum (Al) silicates contained relatively high concentrations of acidic amino acids whereas arctic soils were low in these amino acids; (2) there were smaller amounts of basic amino acids in the tropical soils than in the other soils. This is in agreement with the observation that acidic amino acids are concentrated in particle-size fractions rich in noncrystalline Al compounds (Schnitzer and Kodama 1992). In contrast, the distribution of neutral and sulfur-containing amino acids was similar in all soils. Glucosamine was always present in greater amounts than galactosamine.

Sowden et al. (1977) compared the mean amino acid composition of the soils analyzed with those of algae, bacteria, fungi, and yeasts, and found that the amino acid composition of soils was most similar to that of bacteria. This indicates that in soils microbes play a major role in the synthesis of proteins, peptides, and amino acids from plant and animal residues.

Stevenson (1994) lists the occurrence of the following α -amino (protein) acids in soils:

neutral amino acids: glycine, alanine, leucine, isoleucine, valine, serine, and threonine; *secondary amino acids:* proline and hydroxyproline; *aromatic amino acids:* phenylalanine, tyrosine and tryptophane; *acidic amino acids:* aspartic acid and glutamic acid; *basic amino acids:* arginine, lysine, and histidine.

Other amino acids first detected by Bremner (1967) are: α -amino-*n*-butyric acid, α,ϵ -diaminopimelic acid, β -alanine, and γ -amino-butyric acid. In addition, Stevenson (1994) found the amino acids ornithine, 3,4-dihydroxyphenylalanine and taurine in soils; these amino acids are not normal constituents of proteins. Diaminopimelic acid, which originates from cell wall peptidoglycans of procaryotes, in loamy sand accounted for 0.5% of total amino acid N (Christensen 1996). Most research revealed that there were no great variations in the proportions of amino acids, either among different particle-size fractions (Christensen 1996) or between differently managed soils (Christensen and Bech-Andersen 1989).

Amino sugars in soils

The most prominent amino sugars detected in soils are D-glucosamine and D-galactosamine, with the former occurring in greater amounts (Stevenson 1994). Other amino sugars, detected in relatively small amounts, are muramic acid, D-mannosamine, N-acetylglucosamine and D-fucosamine. There are possibly other amino acids present in soils that have yet to be identified (Stevenson 1994).

Nucleic acid bases in soils and humic substances

Anderson (1957, 1958, 1961) identified guanine, adenine, cytosine, thymine, and traces of uracil in acid hydrolysates of humic acids extracted from Scottish soils. Later, Cortez and Schnitzer (1979) determined the distribution of purines (guanine and adenine) and pyrimidines (uracil, thymine, and cytosine) in 13 soils and humic materials. Concentrations of purines plus pyrimidines ranged from 20.9 to 137.7 $\mu\text{g g}^{-1}$ of dry soil, from 210.8 to 810.0 $\mu\text{g g}^{-1}$ of dry, ash-free humic acids, and from 294.3 to 1086.0 $\mu\text{g g}^{-1}$ of dry, ash-free fulvic acids. Quantitatively, the distribution in soils was: guanine > cytosine > adenine > thymine > uracil. Humic acids were richer in guanine and adenine but poorer in cytosine, thymine and uracil than fulvic acids. The ratio of guanine plus cytosine to adenine plus thymine was > 2 for soils and humic substances. The absence of methylcytosine suggested that the nucleic acid bases extracted were of microbial DNA origin. An average of 3.1% of the total N in agricultural soils, but only 0.3% of the total N in organic soils, was found to occur in nucleic acid bases.

¹⁵N NMR analyses of soils and humic materials

¹⁵N NMR has been used by a number of workers for the analysis of N compounds in peats, plant composts, whole soils, and humic fractions (Preston et al. 1982; Benzing-Purdie et al. 1983, 1986; Almendros et al. 1991; Zhuo et al. 1992, 1995; Zhuo and Wen 1992, 1993; Knicker et al. 1993, 1995, 1996; Knicker and Luedemann 1995; Steelink

C 1994). In a recent review, Preston (1996) noted that in all studies done so far on soils, humic substances, and composts, the ^{15}N NMR spectra are very similar and remarkably simple, consisting of one major peak due to amide/peptide and a few minor signals arising from indoles, pyrroles, and amino N. There has been no indication of the presence of significant reservoirs of unusual N forms. Along the same lines, Zhuo and Wen (1992) reported that in the ^{15}N -NMR spectrum of ^{15}N -labelled humic acid, 86.4% of the total area is due to amide/peptide, 4.3% to aliphatic amines, 3.9% to aliphatic and/or aromatic amines, and only 5.4% to pyrrole N. Similarly Knicker et al. (1993) stated that 85% of the signal intensity in ^{15}N -NMR spectra of ^{15}N -enriched composts and recently formed humic materials is due to amide/peptide and that no signals in the range typical of heteroatomic N compounds were detected. In a more recent study, Knicker and Luedemann (1995) composted ^{15}N -enriched rye grass and wheat for up to 630 days. The composts were characterized periodically by ^{15}N solid-state cross-polarization/magic-angle-spinning (CPMAS) and solution NMR. Most of the detectable N was assigned to amide/peptide structures (80–90%) and the remaining intensities were assigned to amino- and NH_4^+ -N. Less than 5% of the intensity could possibly be ascribed to indole/imidazole/uric acid N. The authors concluded that their ^{15}N -NMR spectra did not reveal any ^{15}N signals that could be ascribed unequivocally to N heterocyclics.

With the exception of Knicker et al. (1993) and Knicker and Luedemann (1995), none of the scientists employing ^{15}N NMR appear to be aware of the wide divergences that exist between chemical and ^{15}N NMR measurements on soils and humic substances. For example, chemical methods indicated a maximum protein content of 40% (Sowden et al. 1977), but ^{15}N NMR of ^{15}N -labelled soils and humic substances revealed a protein content of 85% (Knicker et al. 1993). Similarly, on the basis of chemical techniques measuring UH-N and NH-N, between 27% and 34% of the total N appears to be heterocyclic, compared to only 5% to 10% indicated by ^{15}N NMR. What are the reasons for these discrepancies? A more detailed analysis of the problem shows that:

1. Natural ^{15}N abundance levels in soils and humic materials are low (0.4%) so that direct analysis by ^{15}N NMR is difficult or impossible; also, the gyromagnetic ratio of the ^{15}N nucleus is small, which adds to the difficulties.

2. To overcome these problems, ^{15}N concentrations in soils and humic materials are increased with ^{15}N -labelled salts such as $(^{15}\text{NH}_4)_2\text{SO}_4$, but incubation under these conditions, even for up to 630 days, does not produce the same array of ^{15}N compounds that are normally synthesized in soils and humic materials in the presence of reactive surfaces and over a period of hundreds or thousands of years. During the early stages of incubation there is the microbial synthesis of proteins and of very few heterocyclics.

3. It is likely that in soils and humic materials large numbers, possibly more than hundred different N heterocyclics are formed microbially and/or chemically, so that

^{15}N NMR in its current state of development is unable to resolve this complex mixture.

In an attempt to tackle the problem of very low ^{15}N natural abundance in soils and humic materials, Knicker et al. (1993) ran ^{15}N NMR spectra on soils and humic substances without ^{15}N labelling. They managed to record ^{15}N spectra with tolerable signal-to-noise ratios after accumulating approximately one million transients. Again, most of the intensity was found in the amide/peptide region, but this band was broad and could have covered less intense signals originating from indoles, purines, quinone imines, lactams, carbamates, melanoids, and Maillard products. Other signals with poor signal-to-noise ratios were apparently due to NH groups in guanidine, and aniline derivatives, and to free amino groups of amino acids and amino sugars as well as to substituted amines. These data indicate that only with substantial improvements in instrumental design and procedures, the gulf between results obtained by ^{15}N NMR and chemical and mass spectrometric methods will narrow.

Availability of NH-N

To discover whether N in the NH-N fraction in soils was available to microbes, NH-N fractions from several soils were incubated with a clay soil, a sandy soil, and pure sand (Ivarson and Schnitzer 1979). At pH 7.0, the order of biodegradation in the three media was sand > sandy soil > clay soil. Most of the NH-N was found to be reduced to ammonia by biological activity. Additional evidence for the biodegradability of NH-N has been reported by Keeney and Bremner (1964), Meints and Peterson (1977), Ottow (1978), and Zhuo et al. (1995). Mild chemical oxidation with peracetic acid converted up to 59% of the NH-N fractions from several humic materials to NH_3 and other N gases (Schnitzer and Hindle 1980). The above data show that the NH-N fraction is not inert but can be converted microbiologically and chemically to NH_3 and other N-gases. However, the contribution of NH-N to the N-nutrition of plants is not known.

Chemistry of UH-N

Schnitzer et al. (1983) developed a chromatographic separation procedure that could separate unidentified N from the known. They hoped that this approach would allow them to take an unhindered look at the unidentified N compounds without interference from the known N compounds. They used their procedure initially to examine the UH-N fraction because they thought that it would be easier to work with the UH-N than with the NH-N fraction. Their procedure was as follows:

1. A number of humic and fulvic acids were hydrolyzed for 24 h with hot 6 M HCl;
2. The soluble hydrolyzates were neutralized and the soluble materials separated on Sephadex G-25 gels.

3. The highest molecular weight fractions were further separated on Sephadex G-50 gels, and the second highest molecular weight fractions on Sephadex G-15 gels.

In this manner, several fractions were prepared from the humic and fulvic acids which contained between 97.5% and 98.6% unidentified N, but only 0.84% amino acid N, 0% amino sugar N, and 0.53% ammonia N.

In a subsequent study, Schnitzer and Spiteller (1986) hydrolyzed the fractions with 2 M H₂SO₄. After neutralization of the soluble material, the latter was reduced with NaBH₄, and then acetylated. The resulting acetates were analyzed by capillary gas chromatography/mass spectrometry, and identified by comparing their mass spectra with those of reference compounds of known structures, and with literature data. Eighteen N heterocyclics were identified. These compounds included hydroxy- and oxyindoles, quinolines and isoquinolines, aminobenzofurans, hydroxypiperidines, hydroxy-pyrrolines, and hydroxypyrrolidines. In addition, a number of benzylamines and nitriles were also identified. It is especially noteworthy that Schnitzer and Spiteller (1986) isolated and detected the N heterocyclics without the use of pyrolysis. They realized that it was only a matter of time before additional heterocyclics would be identified in both the UH-N and NH-N fractions.

Analytical pyrolysis of UH-N and NH-N fractions of soils and soil size separates

Schulten et al. (1997a) identified N compounds in the UH-N fractions of two soils by direct, in-source pyrolysis-field ionization/mass spectrometry (Py-FIMS) and Curie-point pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). These N-compounds are listed in Table 3, and their chemical structures are shown in Fig. 1. The following compounds were detected: pyrazole (IVa), imidazole (IIIa), *N,N*-dimethyl-methanamine (Ip), benzeneacetoneitrile (IXe), propanenitrile (Il), and propenenitrile (Ik).

In the NH-N fractions separated from these soils the following N-containing compounds were identified by Py-GC/MS: pyridine (Va), methylpyridine (Vb), pyrrole (IIa), methylpyrrole (IIb), benzeneamine (IXa), benzonitrile (IXb), isocyanomethylbenzene (IXf), methylbenzonitrile (IXd), benzothiazole (XXIa), indole (XVIa), dodecanenitrile (Iv), tetradecanenitrile (Iv), pentadecanenitrile (Iv), and hexadecanenitrile (Iv). Prominent among the compounds identified were N derivatives of benzene (benzeneamine (IXa); benzonitrile (IXb), isocyanomethylbenzene (IXf), benzothiazole (XXIa), and indole (XVIa). It is of note that benzeneamine, benzonitrile, and isocyanomethylbenzene are not detectable in plant and microbial substances examined by Py-GC/MS, but are present in soil samples, humic fractions, and hydrolysis residues. Thus, the NH-N fraction is rich in N-benzene derivatives which appear to be soil-specific.

Leinweber and Schulten (1997) identified the following N heterocyclics by Py-GC/MS in the NH-N fractions of

particle size fractions separated from unfertilized and fertilized soils: Benzothiazole (XXIa), substituted imidazoles (XIa to XIg), pyrrole and substituted pyrroles (IIa to IIh), pyridines (Va, Vb), pyrazole and substituted pyrazoles (IVa to IVg), an isoquinoline derivative (XXa), substituted pyrazines (XVIIa, XVIIb), and piperazine (XVIIIa). In addition, aliphatic and aromatic nitriles (Ik, Il, Iq, IXb, IXd, IXi) and low-mass N compounds, including hydrocyanic acid (Ic), dinitrogen (Id), dinitrogen monoxide (Ii), isocyanomethane (Ig), acetamide (In), and hydrazoic acid (Ih) were also identified.

By contrast, Knicker et al. (1995) believe that the NH-N fraction of SOM consists mainly of refractory peptide-like structures, which cannot be broken up by commonly used degradation methods.

Curie-point pyrolysis-gas chromatography/mass spectrometry of whole soils

Schulten et al. (1995) analyzed a number of whole soils by Py-GC/MS with N-selective detection. Among the N-containing pyrolysis products identified were: pyrroles (IIa to IIh), free (IIIa) and substituted imidazoles (XIa to XIe), pyrazoles (IVa to IVg), pyridines (Va to Vj), substituted pyrimidine (XXIIa), pyrazines (VIa and XVIIa,b), indoles (XVIa to XVIk), methylindazole (XIVa), ketones (XIIIa, XXVIa), N derivatives of benzene (IXa to IXp), alkyl nitriles (Ik, Il, Im, Ip, Iq, Is, It, Iu, Iv, Iw,.) and aliphatic amines (If, Ir, XVIIIa). Several compounds were identified which are normally not detected under the same experimental conditions in pyrolyzates of plants and microorganisms. These are N derivatives of benzene and long-chain alkyl nitriles. A summary of the N compounds identified by Py-FIMS and Py-GC/MS is shown in Fig. 2.

Origins of the major compounds identified

A few comments on the possible origins of the major compounds identified in the pyrolyzates may be appropriate at this point.

Pyrroles

Proline and hydroxyproline release pyrrole and pyrrolidine as major pyrolysis fragments (Irwin 1982; Chiavari and Galetti 1992) and pyrrolidine can also be produced from the pyrolysis of polyglutamic acid (Johnson et al. 1973). In addition, the thermal degradation of glutamine and asparagine produces derivatives of pyrrole (Chiavari and Galetti 1992). Boon and de Leeuw (1987) identified pyrrolidones and pyrrolidine-diones as primary pyrolysis products of proteins, plants, marine sediments, and soil humic acids. Bracewell and Robertson (1984) showed that all pyrroles and acetonitriles produced by the pyrolysis of

Table 3 Molecular weights, elemental analyses, and identities of N compounds in humic acids and soils as determined by Py-FIMS and Py-GC/MS (from Hempfling et al. 1988; Sorge et. al. 1993; Schulten and Schnitzer 1992; Schulten et al. 1995, 1997a; Leinweber and Schulten 1997)

Compound No.	Measured mass	Elemental Composition	Identity	Compound No.	Measured mass	Elemental Composition	Identity
Ia	17	NH ₃	ammonia	IXe	117	C ₈ H ₇ N	benzeneacetonitrile
Ib	18	NH ₄ ⁺	ammonium	IXf	117	C ₈ H ₇ N	isocyanomethylbenzene
Ic	27	HCN	hydrocyanic acid	IXg	119	C ₇ H ₅ ON	4-hydroxybenzonitrile
Id	28	N ₂	nitrogen	IXh	121	C ₈ H ₁₁ N	benzenamine, 2,5-dimethyl
Ie	30	NO	nitrogen oxide	Iu	122	C ₇ H ₁₀ N ₂	heptanedinitrile
If	31	CH ₅ N	methylamine	IVf	124	C ₇ H ₁₂ N ₂	butylpyrazole
Ig	41	C ₂ H ₃ N	isocyanomethane	IVg	124	C ₇ H ₁₂ N ₂	1-ethyl-3,5-dimethyl-pyrazole
Ih	43	HN ₃	hydrazoic acid	XVIIa	124	C ₆ H ₈ ON ₂	2-methoxy-3-methyl-pyrazine
Ii	44	N ₂ O	dinitrogen monoxide	XVIIIa	129	C ₆ H ₁₅ N ₃	1-piperazineethanamine
Ij	45	CH ₃ NO	formamide	XIe	130	C ₅ H ₁₀ O ₂ N ₂	2-ethyl-4,5-dihydroxy-imidazole
Ik	53	C ₃ H ₃ N	2-propenenitrile	XVIIb	131	C ₉ H ₉ N	3-methylindole
Il	55	C ₃ H ₅ N	propanenitrile	XVIc	131	C ₉ H ₉ N	5-methylindole
Im	55	C ₃ H ₅ N	isocyanethane	IXi	131	C ₉ H ₉ N	benzenepropanenitrile
In	59	C ₂ H ₅ NO	acetamide	XIf	132	C ₈ H ₈ N ₂	methylbenzimidazole
Io	59	C ₃ H ₉ N	<i>N,N</i> -dimethyl methanamine	XIVa	132	C ₈ H ₈ N ₂	1H-indazole, 5-methyl-
Ila	67	C ₄ H ₅ N	pyrrole	XIXa	133	C ₈ H ₇ NO	benzoxazole, 2-methyl-
IIla	68	C ₃ H ₄ N ₂	imidazole	XXa	133	C ₉ H ₁₁ N	isoquinoline, 1,2,3,4-tetrahydro
IVa	68	C ₃ H ₄ N ₂	pyrazole	Vi	135	C ₉ H ₁₃ N	pyridine, 2-ethyl-4,6-dimethyl
Ip	69	C ₄ H ₇ N	2-methylpropanenitrile	IXj	135	C ₈ H ₉ NO	methyl-amino-benzaldehyde
Va	79	C ₅ H ₅ N	pyridine	XXIa	135	C ₇ H ₅ SN	benzothiazole
VIa	80	C ₄ H ₄ N ₂	pyrazine	IXk	137	C ₈ H ₁₁ ON	amino-benzene-ethanol
IIb	81	C ₅ H ₇ N	methylpyrrole	XXIIa	140	C ₆ H ₈ O ₂ N ₂	2,4[1H, 3H]-pyrimidine=dione, 3,6-dimethyl
IIc	81	C ₅ H ₇ N	<i>N</i> -methylpyrrole	IXl	145	C ₉ H ₇ ON	benzoacetonitrile
IVb	82	C ₄ H ₆ N ₂	methylpyrazole	XVIId	145	C ₁₀ H ₁₁ N	2,6-dimethylindole
VIIa	83	C ₅ H ₆ N	tetrahydropyridine	XVIe	145	C ₁₀ H ₁₁ N	ethylindole
Iq	83	C ₅ H ₉ N	3-methylbutanenitrile	XIg	146	C ₉ H ₁₀ N ₂	1-ethylbenzimidazole
IIId	83	C ₄ H ₅ NO	hydroxypyrrrole	IXm	149	C ₈ H ₇ NO ₂	hydroxymethoxybenzonitrile
IVc	84	C ₄ H ₈ N ₂	4,5-dihydro-3-methyl=pyrazole	IXn	149	C ₉ H ₁₁ ON	dimethyl-amino-benzaldehyde
Ir	87	C ₃ H ₅ NO ₂	formylacetamide	XVIIb	152	C ₈ H ₁₂ N ₂ O	methoxy-propyl-pyrazine
VIIIa	91	C ₆ H ₅ N	cyanocyclopentadiene	Vj	153	C ₇ H ₇ NO ₃	hydroxy-acetoxy-pyridine
Vb	93	C ₆ H ₇ N	methylpyridine	XXIIIa	157	C ₁₁ H ₁₁ N	ethylquinoline
IXa	93	C ₆ H ₇ N	benzenamine	XVIIf	159	C ₁₁ H ₁₃ N	1,2,3-trimethylindole
Vc	94	C ₅ H ₆ N ₂	aminopyridine	XVIg	161	C ₁₀ H ₁₁ NO	indoleethanol
Xa	94	C ₅ H ₆ N ₂	2-methylpyrimidine	IXo	163	C ₉ H ₉ NO ₂	dimethoxy-benzonitrile
IIe	95	C ₆ H ₉ N	dimethylpyrrole	XXIVa	171	C ₁₂ H ₁₃ N	propylquinoline
IIIf	95	C ₅ H ₅ NO	2-formylpyrrole	XVIh	171	C ₁₂ H ₁₃ N	1H-carbazole, 2,3,4,5-tetrahydro
Vd	95	C ₅ H ₅ NO	3-hydroxypyridine	XVIIi	173	C ₁₁ H ₁₁ NO	methyl-acetyl-indole
IVd	96	C ₅ H ₈ N ₂	dimethylpyrazole	XVIj	175	C ₁₀ H ₉ NO ₂	indole-acetic acid
XIa	96	C ₅ H ₈ N ₂	2,4-dimethylimidazole	XVIIk	175	C ₁₀ H ₉ NO ₂	methyl-indole-carboxylic acid
XIb	96	C ₅ H ₈ N ₂	2-ethyl-1H-imidazole	XXVa	185	C ₈ H ₁₁ NO ₄	dianhydro-2-acetamide-2-deoxyglucose
Is	97	C ₆ H ₁₁ N	pentanenitrile, 4-methyl	XXVIa	186	C ₁₀ H ₈ N ₂ O ₂	diketodipyrrole
XIc	98	C ₄ H ₆ ON ₂	imidazole, 4-methanol	XXVIIa	186	C ₉ H ₆ N ₄ O	pyrazolo [5, 1-c] [1, 2, 4] benzotriazine-8-ol
XId	98	C ₅ H ₁₀ N ₂	4,5-dihydro-2,4-dimethyl-1H-imidazole	IXp	211	C ₈ H ₅ O ₆ N	3-nitro-1,2-phthalic acid
XIIa	99	C ₆ H ₁₃ N	aminocyclohexane	Iv	195–279	C ₁₃ H ₂₅ N-C ₁₉ H ₃₇ N	<i>n</i> -C ₁₃ to <i>n</i> -C ₁₉ alkylnitriles
XIIIa	99	C ₄ H ₅ O ₂ N	2,5-pyrrolidinedione	Iw	220–290	C ₁₄ H ₂₄ N ₂ -C ₁₉ H ₃₄ N ₂	<i>n</i> -C ₁₄ to <i>n</i> -C ₁₉ dialkylnitriles
IXb	103	C ₇ H ₅ N	benzonitrile				
Ve	104	C ₆ H ₄ N ₂	pyridine, 3-nitrile				
Vf	107	C ₇ H ₉ N	dimethylpyridine				
IXc	107	C ₇ H ₉ N	1-amino-3-methylbenzene				
Vg	107	C ₇ H ₉ N	2-ethylpyridine				
VIIb	108	C ₆ H ₈ N ₂	pyrazine, 2,3-dimethyl				
IIg	109	C ₇ H ₁₁ N	2,3,5-trimethylpyrrole				
IIh	109	C ₆ H ₇ NO	2-acetylpyrrole				
IVe	110	C ₆ H ₁₀ N ₂	1,3,5-trimethylpyrazole				
It	111	C ₇ H ₁₃ N	dimethylbutylnitrile				
Vh	111	C ₅ H ₅ NO ₂	dihydroxypyridine				
XVa	113	C ₅ H ₇ NO ₂	aminomethylfuranone				
XVIIa	117	C ₈ H ₇ N	indole				
IXd	117	C ₈ H ₇ N	methylbenzonitrile				

(Continued)

Fig. 1 Chemical structures of N compounds identified in humic acids and whole soils by Py-FIMS and Curie-point Py-GC/MS

three humic acids originated from hydrolyzable amino acids. Substituted pyrroles are formed readily when porphyrin is pyrolyzed; porphyrin is an essential component of the chlorophyll molecule in terrestrial plants (Bracewell et al. 1987).

Imidazole

The pyrolysis of histidine produces derivatives of imidazoles (Irwin 1982). Also, the thermal degradation of grass and soil microorganisms forms imidazoles.

Pyrazoles

The pyrolysis of grass and soil microorganisms produces pyrazol derivatives.

Pyridines

The pyrolysis of α - and β -alanine (Lien and Nawar 1974), polypeptides (Martin et al. 1979), and chitin (van der Kaaden et al. 1984) produces pyridine and alkylpyridines. According to Bremner (1967), pyridine and pyridine derivatives are formed by microbial decomposition of plant lignins and other phenolics in the presence of NH_3 .

Fig. 2 Proposed structures of soil organic N constituents in four mineral soils as derived by Curie-point Py-GC/MS with nitrogen-selective detection. The structures displayed give a qualitative survey of the different classes of N-containing compounds and their distribution to total N (N_t) (Schulten et al. 1995)

Pyrimidines

As has been mentioned earlier, up to 3% of the total soil N occurs in purines and pyrimidines. These compounds are highly polar and cannot be eluted from gas chromatographic columns without prior derivatization. This explains why only one pyrimidine derivative has been identified.

Pyrazines

The pyrolysis of hydroxy-amino acids produces pyrazine and various alkyl pyrazines (Chiavari and Galetti 1992). Pyrazines are also formed by the thermal degradation of dipeptides (alanyl serine and glycyl serine) (Merritt and Robertson 1967) and polypeptides (Martin et al. 1979). Interestingly, Curie-point tandem mass spectrometry of oligopeptides gave diketopiperazines or cyclic dipeptides as major decomposition products and allowed to identify peptide pairs present in complex systems (Voorhees et al. 1994).

Indoles and quinolines

Tsuge et al. (1985) showed by microfurnace Py-GC/MS of tryptophan that indole and indole derivatives substituted in 3-position were formed through C-C bond scissions at the α - and β -positions from the amino group. More recently, Chiavari and Galetti (1992) confirmed that tryptophan releases both indole and 3-methylindole as major pyrolysis products. Rinderknecht and Jurd (1958) have proposed the following rearrangement of the product of the reaction of phloroglucinol with glycine to form 1,3-dihydroxyindole:

Similarly, Hackmann and Todd (1953) showed that the product of the reaction of orthoquinone and a terminal amino group of a protein can rearrange to form an indole protein complex:

N-containing derivatives of benzene

N-containing derivatives of benzene so far identified in soil pyrolyzates include aromatic amines, aromatic nitriles, benzoxazoles, and aromatic nitro-compounds. Of these compounds, only benzeneacetonitrile has been identified in plant and microbial SOM precursors. Phenylalanine releases benzeneacetonitrile as a product of pyrolysis (Chiavari and Galetti 1992). The remaining N derivatives of benzene appear to be soil specific.

Aliphatic amines

The *N,N*-dimethyl-methanamine identified after the thermal degradation of soils also occurs in pyrolyzates of plant and microbial SOM precursors (Schulten et al. 1995).

Alkyl nitriles

Nitriles can be formed from the thermal decomposition of amines by the loss of two hydrogen molecules (Chiavari and Galetti 1992). *N*-Alkyl nitriles have previously been identified in pyrolyzates of kerogens isolated from various marine and lacustrine oil shales (Regtop et al. 1982). They could have originated either, as mentioned previously, from amines, or from the dehydration of amides; these amides being formed either as primary pyrolysis products (Regtop et al. 1982) or as secondary products by reaction of *n*-alkanoic acids with NH_3 (Evans et al. 1985). Derenne et al. (1993) report that *n*-alkyl nitriles were produced by pyrolysis from nonhydrolyzable biomacromolecules from the outer cell walls of green microalgae. It is not known at this time whether such macromolecules exist in cell walls of soil microorganisms or plant roots. The long-chain alkyl nitriles detected by Schnitzer (1984) and Schulten et al. (1997a) appeared to be soil specific.

Theories on the synthesis and nature of N heterocyclics in soils

The numerous theories advanced over a period of many years on the origin and chemical structures of heterocyclic

N compounds in soils (Schnitzer 1984) have focused on N compounds formed by reactions of phenols and quinones with proteins, peptides, amino acids, and NH_3 . The following interaction of glycine with phenol has been described by Piper and Posner (1972):

The amino acid cannot be hydrolyzed by hot acid from N-(p-hydroxyphenyl) glycine, but after oxidation to N-(methylcarboxyl) quinonimine, the amino acid can be split off by alkaline hydrolysis.

Theis (1945) suggested that ϵ -lysylamino groups of proteins react with quinones through covalent bonds in the following manner:

According to Flaig et al. (1975), reactions of phenols with NH_3 , followed by autopolymerization under oxidative conditions, lead to formation of complex N-containing polymers:

The products of these reactions could be the precursors of the aromatic amines and/or the aromatic nitriles identified in soil pyrolyzates (Schulten et al. 1995). An examination of the N compounds identified in soils shows a predominance of a wide variety of methyl and alkyl substitutions. For example, the chain lengths of alkylnitriles range from

C_3 to C_{19} . It is possible that the methyl groups and alkyl chains are remnants of longer aliphatic and olefinic chains linking the different SOM components. The situation with regard to the high alkyl substitution of N compounds in soils resembles that of soil humic substances for which C-C alkyl aromatics have been proposed as major building blocks (Schulten and Schnitzer 1993, 1995; Schnitzer 1994; Schulten 1994, 1995, 1996a,b) linking aromatic rings.

Similarly, heterocyclic N compounds appear to be linked to these building blocks by alkyl chains which would stabilize the former and make them resistant to hydrolysis and microbial degradation. Other mechanisms of stabilization are intensive crosslinking with other SOM components and/or interactions with metals and clay minerals.

Support for the presence of significant NH-N components in humic substances and soils comes from the recent work of Leinweber and Schulten (1997), who showed that these compounds had a strong resistance to hot acid hydrolysis plus high thermal stability. Thus, differences between UH-N and NH-N fractions may be due to different strengths of bonds and crosslinks; the latter appear to be stronger in the NH-N than in the UH-N fractions.

Structural concept for N compounds in SOM and whole soils

Recently an improved total humic substance (THS) model has been proposed (Schulten and Schnitzer 1997). This model has the elemental composition $\text{C}_{305}\text{H}_{299}\text{N}_{16}\text{O}_{134}\text{S}_1$, elemental analysis of 57.56% C, 4.73% H, 3.52% N, 33.68% O, 0.5% S, and a molecular weight of $6364.814 \text{ g mol}^{-1}$. The elementary analysis of this THS model is close to that of naturally occurring humic acids (Schnitzer 1978). As shown in Fig. 3a, the sizes of the structural voids of the two-dimensional (2D) model are ample for the occlusion of peptides, polysaccharides, water, etc. The draft structure contains 5 aliphatic and 21 aromatic carboxyl groups, 17 phenolic and 17 alcoholic groups, 16 nitrogen functions, 7 quinonoic and ketonic groups, 3 methoxyl groups, and 1 aromatic sulfur. The distribution of these functional groups is in reasonable agreement with experimental data. Of particular importance are the positions of the 16 N atoms which are indicated by their atom numbers. Starting with N atoms, numbers 25 and 26 stand for pyrazole, 94 for indole, 192 for pyrrole, 220 for benzothiazole, and 432 for pyridine; the corresponding aromatic nitrogens are shown in Fig. 3a. In addition, three nitriles, four aliphatic and two aromatic amines and acetamide are displayed. At this stage of high total energy (and low geometry optimization) of the preliminary THS structure, ten hydrogen bonds are formed; the nitrogen atoms numbered 145, 453 and 638 participate in five of these (Schulten and Schnitzer 1997). The color 2D plot of the THS structure in Fig. 3b illustrates the space requirements of the 755 atoms and gives a first impression of the distribution and frequency of C, H, N, S, atoms and shows above all the high oxygen content of the structural model. Moreover, the presence of N heterocyclics such as

Fig. 3a,b Draft of an improved 2D model structure of total humic substance (THS; $C_{305}H_{299}O_{134}N_{16}S$; 755 atoms) created by using the drawing tools in the workspace of the PC screen. The handdrawn preliminary structure was improved using HyperChem software. The structure is displayed in: **a** *Sticks*; the 16 nitrogen atoms in the 2D structure are labelled by atom numbers, and **b** *Disks*; element colors are carbon (cyan), hydrogen (white), oxygen (red), nitrogen (blue) and sulfur (yellow). The presentation is performed using the ChemPlus software (*Disks and Bonds*) (Schulten and Schnitzer 1997)

Fig. 4 Geometrically optimized 3D structure of a model of soil organic matter using THS with occluded trisaccharide, hexapeptide and 3% water content ($C_{349}H_{401}N_{26}O_{173}S_1$, 950 atoms) is shown. The element colors and presentation (ChemPlus software) are as described in Fig. 3 (Schulten and Schnitzer 1997)

indoles, pyrroles, benzothiazole, pyridines, etc. proposed earlier as essential SOM building blocks by Schulten et al. (1995) is indicated in Fig. 3b.

The basic THS model was further developed into a model of SOM with a water content of 3% and is displayed in the 3D color plot in Fig. 4 (Schulten and Schnitzer 1997). The elemental composition of $C_{349}H_{401}N_{26}O_{173}S_1$, elemental analysis of 54.02% C, 5.21% H, 4.69% N, 35.67% O, 0.41% S and a molecular weight of $7760.154 \text{ g mol}^{-1}$ were determined. The dimensions of the smallest rectangular box enclosing this complex are: $x = 3.25 \text{ nm}$; $y = 2.36 \text{ nm}$; $z = 4.21 \text{ nm}$ and give a rough estimate of 32.29 nm^3 volume for this structure.

Trapped with this structure are a typical soil hexapeptide (1Asp2Gly3Arg4Glu5Ala6Lys; zwitter ion, $C_{26}H_{46}O_{11}N_{10}$) and a trisaccharide ($C_{18}H_{32}O_{16}$), as well as 12 water molecules (H_2O_{12}). In general, trapping and covering of biological molecules in the THS voids (see arrows) and immobilization by hydrogen bonds (Schulten

and Schnitzer 1997) appear to be crucial SOM properties. In order to find the best conformation for the SOM complex of 15 molecules and 950 atoms, geometry optimization (and thus energy minimization) was performed using molecular mechanics calculations, which gave a total energy of $2171.49 \text{ kJ mol}^{-1}$ at an energy gradient of $0.20 \text{ kJ mol}^{-1} \text{ nm}^{-1}$ (software HyperChem, release5, *features in italics*; Hypercube, Inc., 1115 N.W. 4th Street, Gainesville, Florida, U.S.A; Email: info@hyper.com). Using an all atom force field in this mode (*All Atoms, MM+*), the distribution of the calculated total energy was determined. The results are: bond stretching = $165.67 \text{ kJ mol}^{-1}$; angle bending = $1084.99 \text{ kJ mol}^{-1}$; dihedral torsions = $861.94 \text{ kJ mol}^{-1}$; van der Waals interactions = $320.27 \text{ kJ mol}^{-1}$; bond stretch and angle bending cross term = $25.25 \text{ kJ mol}^{-1}$; and the negative term of electrostatic energy of $-286.62 \text{ kJ mol}^{-1}$. The latter is not due to electrostatic charge-charge interaction but comes from defining a set of bond dipole moments associated with polar bonds. Schulten and Schnitzer (1997) suggest on the basis of a recent simulation experiment with polypeptides that a portion of the proteinaceous materials (proteins, polypeptides, peptides, and amino acids) in soils is trapped in the voids of the three-dimensional HA structure but that a greater portion of these compounds is either physically or chemically retained by the HA surface.

Quantitative structure-activity relationships (QSAR) can be calculated and are attempts to correlate molecular structure, or properties derived from the molecular structure, with a specific kind of chemical or biochemical activity (*ChemPlus*). The molecular properties used in the correlations relate as directly as possible to the key processes taking place in the sites of target activity, and are of particular interest in agricultural and environmental chemistry. The empirical calculations of the geometrically optimized SOM complex described above gave the following results. The approximate, solvent-accessible surface area was found by a fast calculation mode to be 740.35 nm^2 . Using the more accurate, but time-consuming, grid method, the surface area was determined as 968.16 nm^2 . Additional data were: solvent-accessible surface-bounded volume = 93.83 nm^3 ; refractivity = 1.74 nm^3 ; polarizability = 0.72 nm^3 ; $\log P = 124.80$; molecular mass = $7760.15 \text{ g mol}^{-1}$. The hydration energy of the trapped hexapeptide AspGlyArgGluAlaLys was determined as $2628.01 \text{ kJ mol}^{-1}$. Other options allow us to determine van der Waals surface areas and van der Waals-surface-bounded molecular volumes, as well as atomic partial charges.

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