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Structural Modification of Lignin in Peat during Peat Formation at Tropical Swamp

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Abstract

Peat samples from typical tropical peat swamp around Narathiwat Province, southern Thailand, were collected from various depths of peat profile (from surface to 210 cm depth), of which dates were established using an accelerator mass spectrometry from 0 to 2600 years BP. The samples were analysed to discuss changes in structural characteristics of lignin in peat samples during peat formation. The samples were fractionated based on pH adjustment into dilute alkali soluble (AL_{sol}) and insoluble (AL_{insol}) fractions. Only glucosyl and xylosyl residues were detected in AL_{insol} fraction, but no monosaccharide in AL_{sol} fraction. Lignin contents of AL_{insol} fraction of the peat samples ranged from 73.0 to 83.3%. Lignin was significantly modified during peat formation. The progress of condensation reactions, introduction of carboxyl groups and partially loss of methoxyl groups, cleavage of arylglycerol-β-aryl ether linkage, were confirmed by alkaline nitrobenzene oxidation, functional group analysis and analytical ozonation, respectively, due to the microbial activities.

Lignin of the peat samples was isolated by Björkman's procedure to discuss structural feature in detail using spectrometrical procedures as the first trial in the field of geochemistry. The signals around 6.6 and 6.9 ppm in ^{1}H-NMR were considerably weak compare with those of lignin from fresh wood, suggesting high condensation. The most peaks originating from lignin of fresh wood were also detected in the FTIR spectrum. The ionization difference (ΔE_i) spectrum clearly showed the presence of unconjugated and conjugated phenolic hydroxyl groups. Thus, lignin in peat was modified significantly by microbial activities. It is expected that further modified fragments of lignin would be soluble in water and flow-out to ocean through rivers.

Discipline: Biomass transformation
Additional key words: analytical ozonation, arylglycerol-β-aryl ether linkage, biological modification of lignin

Introduction

The deposits of peat underneath tropical peat swamp forests are among the world's largest sink of organic carbon. Tropical peat swamp forests account for approximately 38×10^6 ha in the world, and for the carbon sink of $6.3–14.8\times10^{10}$ ton[19]. More than 20×10^6 ha of tropical peat swamp, which is about 53% of all known tropical peat resources in the world, are distributed upon marine clay containing pyrite (FeS_2) surrounding coastal area in Southeast Asia (Sumatra Island; 9.7×10^6 ha, Kalimantan Island; 7.9×10^6 ha, Malay Peninsula; 1.0×10^6 ha)[9]. However, the areas are threatened by large scale deforestation, drainage for land use change to agricultural land and bush fire, are causing enormous carbon dioxide emission.

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The pH of peat deposit was nearly neutral (6.4–7.2) because carboxyl groups in peat form salt with alkaline metal or alkaline earth metal. However, when swamp water covering peat deposit was drained off to be developed in agricultural area or oil palm plantation in the middle of 1970's, which has been reported by Saito et al.[40], peat deposit was decomposed quickly and soil pH drops seriously to lower than 3, because of oxidation of pyrite to H_2SO_4 in marine clay[38,40]. Total area of tropical peat swamp at Narathiwat Province in southern Thailand is about 7×10^4 ha, Bacho peat swamp is the typical developing peat swamp[38]. According to observation data from 1973 to 2005 at the Narathiwat Meteorological Station, the mean annual temperature was approximately 27ºC and the mean annual rainfall was 2,560 mm.

It is well documented that precursors of peat are mainly terrestrial higher plants[5]. Peat is a heterogeneous mixture of more or less biologically modified plant material, and is composed of a skeleton of alkyl and aromatic moieties of which major functional groups are carboxylic acids, phenolic and alcoholic hydroxyls, ketone and quinoid groups[47]. The aromatic units would derive from biologically modified lignin[44]. Lignin would be dominant in peat, because lignin is one of major component of vascular plant tissues as the origin of peat organics and significantly low degradation rate by microorganisms[21,48]. Therefore making clear structural modification of plant cell wall components, especially lignin, during peat formation, would be essential to develop sustainable management systems for these relatively fragile ecosystems. In addition, lignin fragment in peat is soluble in water by further biological modification, and flow out to ocean as dissolved organic matter (DOM), which would be important in global carbon circulation.

Most of organic matter in not only peat but also in soil would originate from lignin in plant residue, especially biologically modified lignin. Lignin is defined as a radical coupling polymer of *p*-hydroxycinnamyl alcohols, of which radicals are caused by peroxidase and/or laccase, and characterised as random polymer with the presence of arylglycerol-β-aryl ether linkage as the major intermonomer linkage. Thus, lignin can be modified biologically from dehydrogenation of phenolic hydroxyl groups by only peroxidase families.

There are so many papers on biodegradation of lignin[28]. However, most of reports are confined to the analysis of low molecular weight degradation products including the degradation pathway using lignin model compounds. There are only few suggestions for the structural feature of high molecular weight residual lignins[7,18]. It need scarcely be said that circumstantial structural investigation of such high molecular weight residual lignin is essential to understand nature of peat samples.

Although the isolation of lignin is indispensable to investigate structural feature of lignin in peat samples in detail, lignin had not been isolated previously. It was limited that chemical feature of lignin was investigated by spectrometric procedures such as cross polarization and magic angle spinning (CP/MAS) solid-state ^{13}C-NMR[11,16,39] and Fourier transform infrared (FTIR)[5,39], analytical pyrolysis[37] and some chemical analyses as oxidative degradation using cupric oxide (CuO) of original peat samples.

CuO oxidation, but not alkaline nitrobenzene oxidation, is widely applied to investigate aromatic moieties of lignin in the field of geochemistry, palaeogeography and so on[5,14,15,29,45,46,48], to avoid production of by-products from nitrobenzene[14]. Alkaline CuO oxidation gives significant amount of *p*-hydroxyacetophenones such as acetovanillon and acetosyringone[14,15,46], and total yield of monomeric oxidation products is considerably lower than that by alkaline nitrobenzene oxidation because of different reaction mechanism between both the procedures[6,22].

In this study, peat samples collected from tropical peat swamps at Narathiwat Province, southern Thailand, were characterised by analytical methods used in plant cell wall science instead of procedures used in soil organic chemistry. An alkaline nitrobenzene oxidation[22], an analytical ozonation[1], methoxyl group[13] and acidic groups determination (carboxyl and phenolic hydroxyl groups) by diazomethane methylation[20] were applied to investigate aromatic composition, intermonomer linkages, the modification of aromatic moiety and the introduction of acidic groups of lignin in peat samples, respectively. In addition, lignin was isolated from peat sample according to Björkman's procedure[3] to discuss in detail structural feature of lignin in peat and to understand process of modification of lignin during peat formation by spectroscopic procedures, such as ^{1}H-NMR, FTIR and UV spectroscopy. The discussion of chemical structural feature of lignin using isolated lignin is the first project in such field, information and procedures used in this paper would be expected to contribute on the field of geochemistry and palaeogeography.

Materials and methods

1. Peat sample

Peat samples were collected using a core sampler from the Bacho tropical peat swamp (6°30'N, 101°30'E) at Narathiwat Province, southern Thailand (Fig. 1), which was developed in the middle of the 1970's as oil palm plantation by the drainage of swamp water[40]. The samples were fractionated into 0–30, 30–60, 60–90, 90–120, 120–150, 150–180, and 180–210 cm depths. The ages of the samples at the depth of 15, 45, 75, 105, 135, 165 and 195

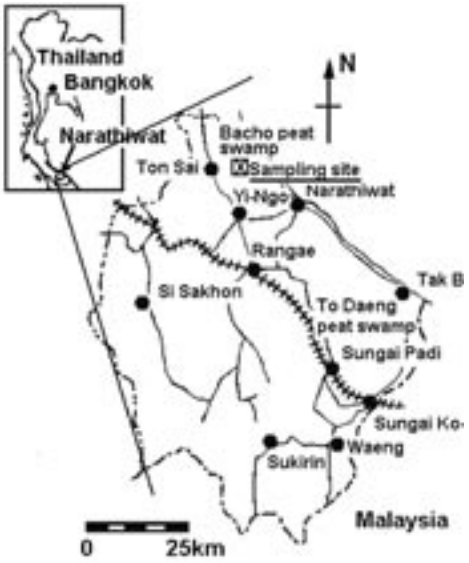

Fig. 1. Sampling site in southern Thailand
Peat samples were collected from developed area at Bacho peat swamp.
☒: Sampling site (6º30'N, 101º30'E).

cm were established using an accelerator mass spectrometry (AMS) at the Research Center for Nuclear Science and Technology, the University of Tokyo, as 0, 1,400, 1,900, 2,000, 2,300, 2,470 and 2,600, respectively.

The peat sample was air-dried and ground using a Wiley mill to pass 420 μm sieves, then extracted successively with water overnight at 40ºC, followed with 0.1M NaOH overnight at 30ºC with shaking. The residue, which is less biological modification fraction, was neutralized with 4M HCl, and then washed repeatedly with H_2O till pH 5 as the AL_{insol} fraction. The supernatant, which is further modified fraction by the introduction of significant amount of hydrophilic functional groups, was acidified to be pH 2 with 4M HCl, and the precipitate was recovered by centrifugation (10,000 rpm, 10 min). The precipitate was washed to be free from Cl^- and freeze dried as the AL_{sol} fraction.

The peat samples collected from 0–30 and 90–120 cm depths were freeze dried and ground using a Wiley mill to pass 420 μm sieves. The samples were extracted with boiling 80% (v/v) ethanol for 1 h (three times) then with water overnight at 40ºC. The extract-free peat sample was dried over P_2O_5 in a vacuum oven overnight at 40ºC, then finely ground for 72 h using a stainless steel vibratory ball mill (VS-2 type, Irie-Shokai Co. Ltd.) without any solvent with cooling by water flow[30] and lignin was isolated according to Björkman procedure[3].

2. Analyses of neutral sugar and lignin

Neutral sugar compositions of AL_{insol} and AL_{sol} fractions were analysed by an alditol acetate method for sulphuric acid hydrolysates[4]. Lignin content was determined gravimetrically by Klason procedure (TAPPI Standard T 222om-88). Lignin contents were corrected for ash by combustion of the Klason lignin for 3 h at 700ºC, and for protein content (nitrogen content × 6.25) by nitrogen content determination using a CHN micro analysis (Perkin Elmer elemental analyzer: PE 240 CHN). The aromatic composition of peat lignin was examined using an alkaline nitrobenzene oxidation as reported by Iiyama and Lam[23] in stead of CuO oxidation which is used widely in the fields of geochemistry, palaeogeography and so on[14,15]. An analytical ozonation was carried out according to the scheme reported by Akiyama et al.[1] to investigate intermonomer linkages between phenylpropane units of lignin. Acidic groups (phenolic hydroxyl and carboxyl groups) were quantified by the determination of methoxyl group contents after methylation with diazomethane followed by saponification with 0.5 M NaOH overnight at 40ºC[20].

Methoxyl group content was determined by a procedure developed by Goto et al. [13]. Sample (20 mg) was soaked in 5 ml of concentrated HI (57%). The mixture was kept in a heating block for 30 min at 130ºC and then cooled with ice bath. Iodoethane was added as an internal standard, followed by 5 ml of CCl_4. After mixing well, an aliquot (1 ml) was taken from the organic layer and dried with anhydrous sodium sulphate. Iodomethane as a product was analysed using a CP-Sil 13 CB capillary column gas chromatography on a Shimadzu GC-17A system equipped with a flame-ionization detector. The injector and detector temperatures were 200ºC and 230ºC, respectively, and column temperature was 40ºC for 5 min, then programmed at 10ºC min^{-1} to 180ºC.

The Björkman lignin (around 100 mg) was acetylated by acetic anhydride with catalytic amount of dry pyridine overnight at room temperature with stirring. The acetylated sample (10 mg) was dissolved in 1 ml of chloroform-d_1 ($CDCl_3$) and recorded ^{1}H-NMR spectrum using a JEOL JNM-A 500 spectrometer. Fourier transform infrared (FTIR) spectrum of the Björkman lignin was measured as a KBr disc on an FT/IR-615 Spectrometer (JASCO, Japan). Unconjugated phenolic hydroxyl group of the lignin was determined by an ionization difference (ΔE_i) spectrum procedure[12] using methlycellosolve-water (1:1, v/v) and methlycellosolve-1M NaOH (1:1, v/v) as solvents. The spectra were recorded on a Hitachi U-3010 spectrophotometer.

Results and discussion

1. Neutral sugar composition

Neutral monosaccharides released by sulphuric acid hydrolysis from AL_{insol} fraction were xylose and glucose, and no monosaccharide was detected from AL_{sol} fraction (Table 1). Xylose would be droved from xylan, which are major components of non-cellulosic cell wall polysaccharide (hemicellulose) in woody and non-woody angiosperm tissue[36]. Total yield of monosaccharides was much lower than that of fresh analogues (Table 1). Significant portion (70–90%) of cell wall polysaccharides of leaf litter was lost after one year mulching[24]. Iiyama and co-workers[21] reported that total yield of neutral monosaccharide of buried hardwoods for 6,000–10,000 years was 15–20% of those from sound wood, probably due to anaerobic atmosphere immediate after buried. Cell wall polysaccharides of plants litter are quickly decomposed, while lignin and polysaccharides covalently associated with lignin resist significantly to biological degradation[21,23,26]. The neutral monosaccharide detected from AL_{insol} fraction would be released from the cell wall polysaccharides associated chemically with lignin.

2. Characterization of peat lignin

Lignin content of the extract free sample was determined by Klason procedure, which affords an insoluble residue from hydrolysis with sulphuric acid digestion. The method could not discriminate only the true lignin, but also other unhydrolysable and/or acid condensed fragments during sulphuric acid treatment, which would be formed during peat formation by microbial activities[2,25,41]. Therefore, Klason procedure would overestimate lignin content. The unhydrolysable residues of the extract free peat samples ranged from 73.0 to 83.3% and from 68.7 to 79.4% for AL_{insol} and AL_{sol} fractions, respectively. There was no significant difference among the peat samples from different depth (Table 1). The significantly high contents of unhydrolysable residues (Fig. 2) would be due to rapid loss of most of cell wall polysaccharides, and significant resistance of lignin to microbial decomposition[16,23]. The peat sample collected from 90–120 cm depth was unfortunately not enough amounts for the above analyses because of preparation of lignin by Björkman procedure.

3. Spectrometric characteristics of peat lignin

There is no spectral information of lignin in peat samples except FTIR[5,39] and ^{13}C-CP/MAS spectra[11,16,39] of

Table 1. Neutral sugar composition, content of unhydrolysable residues by Klason treatment, and structural characteristics of lignin of the extract-free peat samples collected from various depths

Fraction	Peat profile depth (cm)	Yield of fraction (wt%)	Neutral sugar content[a]		Lignin content[b] (wt%)	Methoxyl content of lignin determined for		Alkaline nitrobenzene oxidation		
			Xylosyl residue (wt%)	Glucosyl residue (wt%)		Klason residue mol (200 g lignin)$^{-1}$	Sample mol (200 g lignin)$^{-1}$	Total yield mol (200 g lignin)$^{-1}$	Acid /aldehyde[c]	Syringyl /guaiacyl[d]
Al_{insol}	0–30	64.4	0.5	4.8	80.6	0.25	0.25	0.04	0.38	0.60
fraction	30–60	50.0	0.6	6.3	76.2	0.35	0.30	0.06	0.38	1.09
	60–90	59.1	1.2	12.3	73.0	0.54	0.55	0.11	0.31	0.89
	120–150	65.1	0.5	3.7	83.1	0.32	0.30	0.06	0.66	0.84
	150–180	67.1	0.6	3.7	81.9	0.33	0.33	0.08	0.59	1.18
	180–210	79.3	ND	1.6	83.3	0.21	0.21	0.04	0.31	1.13
Al_{sol}	0–30	29.2	ND	ND	76.4	0.08	0.12	0.02	0.47	0.86
fraction	30–60	33.4	ND	ND	79.4	0.12	0.14	0.03	0.56	1.14
	60–90	17.1	ND	ND	79.2	0.14	0.16	0.03	0.47	1.49
	120–150	28.0	ND	ND	74.4	0.09	0.11	0.03	0.95	1.51
	150–180	14.6	ND	ND	68.7	0.06	0.07	0.01	0.99	1.48
	180–210	9.4	ND	ND	71.4	0.02	0.03	ND	0.67	2.72

a): Detected and quantified by an alditol acetate procedure. Other sugar residues than glucosyl and xylosyl residues were negligible.
b): Klason lignin corrected by ash and protein content.
c): Molar ratio of hydroxybenzoic acids (vanillic and syringic acids) to hydroxybenzaldehydes (vanillin and syringaldehyde) of alkaline nitrobenzene oxidation products.
d): Molar ratio of syringyl (syringaldehyde and syringic acid) to guaiacyl (vanillin and vanillic acid) nuclei of alkaline nitrobenzene oxidation products.
ND: Not detected.

original peat samples, because previous papers analysed peat samples without isolation of lignin. In this paper, lignin was isolated by the extraction with neutral organic solvents from finely ground extract-free peat sample using a vibratory ball mill (Björkman's procedure[3]) to avoid changes in chemical characteristics of lignin during isolation (Table 2).

The yields of Björkman lignin prepared from the extract-free peat sample were 64–66% of the unhydrolysable residues of Klason procedure. Nitrogen content of the Björkman lignins was 0.50 and 0.42%, which were equivalent to 3.1 and 2.6% as protein, for Björkman lignins prepared from peat samples collected at the depths of 0–30 and 90–120 cm, respectively. In addition, neutral sugar contamination was less than 3% in the both Björkman lignins. Prior to the measurement of ^{1}H-NMR spectrum, the Björkman lignin was acetylated as similar as lignin from fresh plant cell walls. The acetylated lignin dissolved easily in $CDCl_3$. The signals assigned to protein and neutral sugars would not appear in ^{1}H-NMR spectrum of the Björkman lignin (Fig. 3), because of significantly low contamination of protein and neutral sugars. The signals around 6.6 and 6.9 ppm in ^{1}H-NMR, which are assigned to aromatic protons of syringyl and guaiacyl nuclei, respectively[33], are considerably weak compared with those of lignin from fresh wood, suggesting the motion of aromatic nuclei is strongly limited by high condensation[32]. The intense signal around 1.2 ppm may suggest methyl proton of fatty acids covalently linked with lignin together with methyl proton generated by the cleavage of aromatic nuclei.

Structural characteristics of the Björkman lignin prepared from peat sample were also discussed by FTIR (Fig. 4) and UV (Fig. 5) spectroscopy. The FTIR spectrum was differentiated numerically to make clear the peak positions. The Björkman lignin showed all peaks appeared in Björkman lignin from fresh angiosperm wood, that is, at 1,712 (-COOH), 1,602 (aromatics), 1,508 (aromatics), 1,460 (methyl), 1,420 (aromatics), 1,375 (aromatics substituted with OH group), 1,325 (syringyl), 1,267 (guaiacyl),

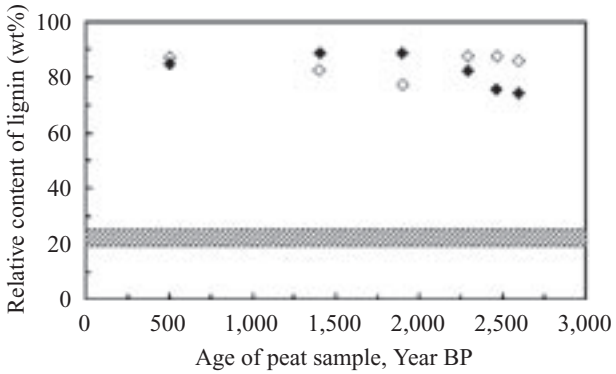

Fig. 2. Level of relative content of lignin in extract-free peat samples collected from various depths
◇: AL_{insol} fraction, ◆: AL_{sol} fraction.
▨: Level of lignin content in fresh tissues of vascular plants.

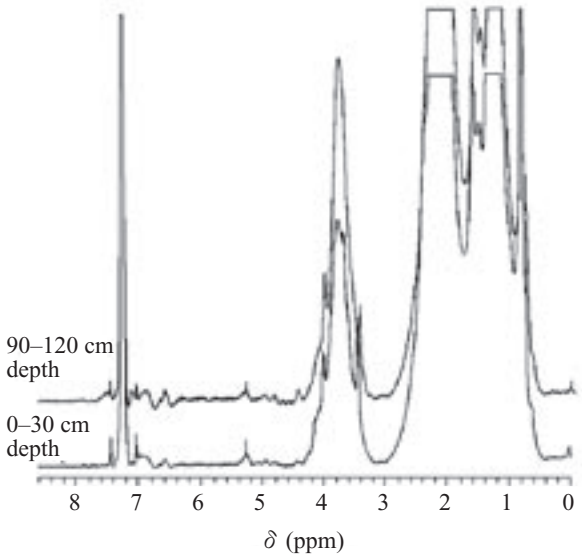

Fig. 3. ^{1}H-NMR spectra of lignins isolated with Björkman's procedure from extractive-free peat samples collected at 0–30 cm and 90–120 cm depths

Table 2. Structural characteristics of lignins isolated from extract-free peat samples collected from 0–30 cm and 90–120 cm depths with Björkman's procedure

Peat profile depth (cm)	Ozonation mol (200 g lignin)$^{-1}$	Methoxyl group content mol (200 g lignin)$^{-1}$	Alkaline nitrobenzene oxidation		
			Total yield mol (200 g lignin)$^{-1}$	Acid /aldehyde[a)]	Syringyl /guaiacyl[b)]
0–30	80.6	0.23	5.45	0.57	0.66
90–120	76.2	0.30	8.59	0.46	0.68

a): Molar ratio of hydroxybenzoic acids (vanillic and syringic acids) to hydroxybenzaldehydes (vanillin and syringaldehyde) of alkaline nitrobenzene oxidation products.
b): Molar ratio of syringyl (syringaldehyde and syringic acid) to guaiacyl (vanillin and vanillic acid) nuclei of alkaline nitrobenzene oxidation products.

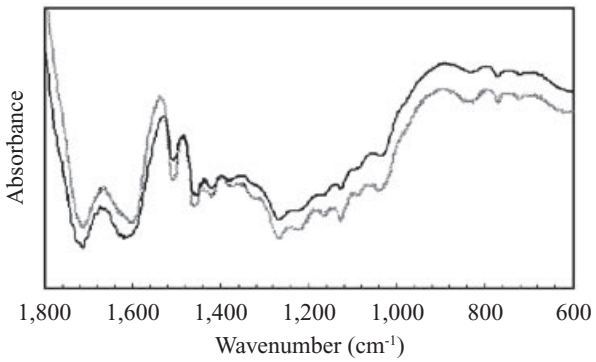

Fig. 4. FTIR spectra of lignins isolated with Björkman's procedure from extract-free peat samples collected at 0–30 cm and 90–120 cm depths and numerical differentiation spectrum to identify peak position
—: 0–30 cm depth, ╍: 90–120 cm depth.

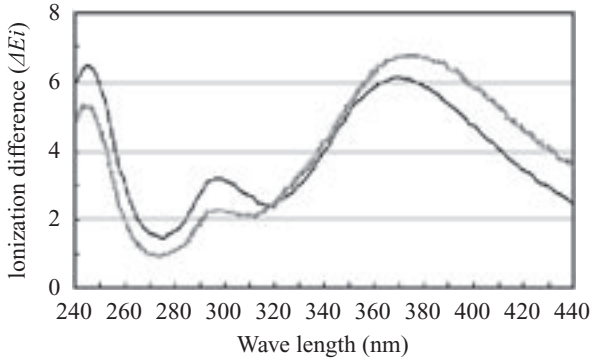

Fig. 5. Ionization difference (*ΔEi*) spectra of lignins isolated with Björkman's procedure from extract-free peat samples collected at 0–30 cm and 90–120 cm depths
—: 0–30 cm depth, ╍: 90–120 cm depth.

1,223 (syringyl substituted with –CO group), 1,126 (syringyl CH), 1,033 (guaiacyl CH), and 831 cm^{-1} (syringyl CH), which were assigned by Kawamura and Higuchi[27], Lam et al.[31] and Sun et al.[42].

Ionization difference (ΔE_i) spectra of the Björkman lignins isolated showed around 295 and 370 nm assigned to unconjugated and conjugated phenolic hydroxyl groups, respectively[10]. Both results from FTIR and ΔE_i spectra clearly indicated the presence of high level of acidic groups and aromatic moieties in the peat Björkman lignin.

4. Aromatic composition and intermonomer linkages

These qualitative results were also supported by an alkaline nitrobenzene oxidation analysis. Hedges and Ertel[14] recommended an alkaline CuO oxidation procedure to investigate aromatic composition of lignin in the field of geochemistry. However, CuO oxidation produces significant amount of *p*-hydroxyacetophenones such as acetovanillon and acetosyringone[14,15,46], and total yield of oxidation products is much lower than that by alkaline nitrobenzene oxidation, because of different reaction mechanism between the both procedures[6,22]. Alkaline nitrobenzene oxidation should be preferred to discuss aromatic composition in this study.

Alkaline nitrobenzene oxidation of the extract-free peat samples collected from various depths gave syringaldehyde and vanillin as major products and significantly wide range of molar ratios of syringyl to guaiacyl nuclei (S/V) (Table 1). The presence of syringyl and guaiacyl nuclei suggests that the origin of peat would be woody and/or non-woody angiosperm, and this suggestion agreed well with the results of neutral monosaccharide composition of sulphuric acid hydrolysate of cell wall polysaccharides (Table 1). Total yields of alkaline nitrobenzene oxidation based on lignin of peat samples were significantly low (Fig. 6). It is well documented that wood samples decayed by microorganisms give lower values of total yield of alkaline nitrobenzene oxidation products than those of fresh plant cell walls[21,23], because of significant condensation by radical coupling reaction due to the activities of lignin peroxidase[17, 23,25,43].

In general, the molar ratio of hydroxybenzoic acids (sum of *p*-hydroxybenzoic, vanillic and syringic acids) to hydroxybenzaldehydes (sum of *p*-hydroxybenzalde, vanillin and syringaldehyde) (Acid/Ald ratio) of alkaline nitrobenzene oxidation products increases significantly with the progress of biological modification of lignin[23,48]. Lignin in fresh tissues of vascular plants gives the Acid/Ald ratio of 0.05–0.10[5,22,39]. The Acid/Ald ratio of the AL_{insol} fractions ranged from 0.31 to 0.66 and those of the

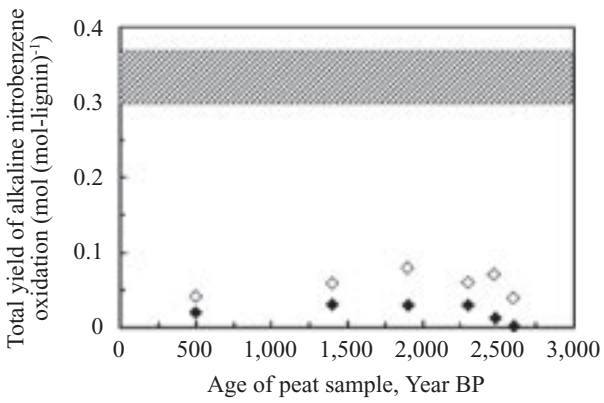

Fig. 6. Level of total yield of alkaline nitrobenzene oxidation products of extract-free peat samples collected from various depths
◇: AL_{insol} fraction, ◆: AL_{sol} fraction.
▨: Level of lignin content in fresh tissues of vascular plants.

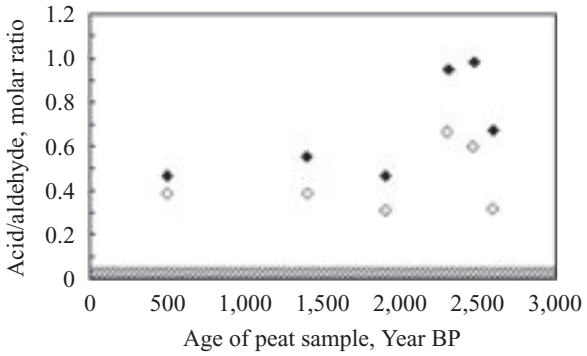

Fig. 7. Level of Acid/Ald molar ratio of alkaline nitrobenzene oxidation products of extract-free peat samples collected from various depths
◇: AL_{insol} fraction, ◆: AL_{sol} fraction.
▨: Level of lignin content in fresh tissues of vascular plants.

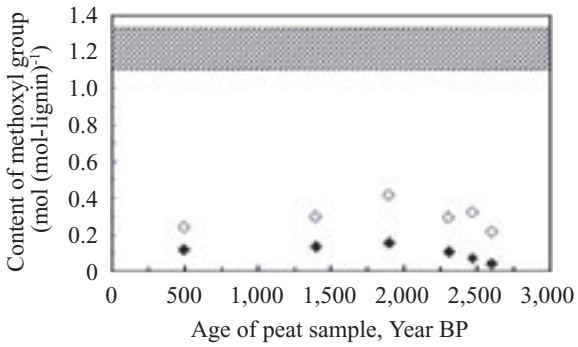

Fig. 8. Level of content of methoxyl groups of extract-free peat samples collected from various depths
◇: AL_{insol} fraction, ◆: AL_{sol} fraction.
▨: Level of lignin content in fresh tissues of vascular plants.

AL_{sol} fractions varied from 0.47 to 0.99 (Table 1). These values were much higher than those of sound woods (Fig. 7), suggesting that oxidative cleavage of the C_α-C_β of lignin side chain and introduction of acidic group during peat formation[8,23].

The presence of arylglycerol-β-aryl ether intermonomer linkages, which is the major intermonomer linkage concerning to the definition of lignin, was confirmed clearly in peat samples by an analytical ozonation. Erythronic and threonic acids are produced by an analytical ozonation from erythro- and threo-forms of arylglycerol-β-aryl ether intermonomer linkages of lignin, which are the most abundant intermonomer linkage of lignin[34]. Total yields of erythronic and threonic acids ranged from 0.01 to 0.03 mol (200 g lignin)$^{-1}$, which was about one tenth of those of sound hardwood lignins. The low yield of analytical ozonation products suggests that the cleavage of arylglycerol-β-aryl ether intermonomer linkages did occur significantly during microbial modification. Some condensation reactions of lignin such as biphenyl formation (5-5' or 6-5' linkages) linkage and diphenyl methane formation (α-1 linkage) during microbial modification were expected. The structure formed by the former condensation does not affect at all on the production of erythronic or threonic acids by ozonation, but erythronic or threonic acids are not produced after the formation of the α-1 linkage.[35] However, the condensation to form the α-1 linkage would not be significant to give huge reduction of the yield of erythronic and threonic acids.

The molar ratio of erythronic to threonic acids (E/T ratio) originating from erythro- and threo-forms of arylglycerol-β-aryl ether linkages, respectively, was discussed. The E/T ratios of the extract free peat samples collected from various depths ranged from 1.0 to 1.3, and did not changed with the depth of sampling. This result suggests that there were no significant differences during biological modification of arylglycerol-β-aryl ether intermonomer linkages of lignin between erythro- and threo-forms.

Methoxyl group content of the extract-free peat samples collected from various depths was only 20–50% of those of sound wood lignins (Fig. 8). Previous studies on wood decay indicated that the methoxyl group content of lignin decreases with the progress of decay[17]. Methoxyl contents of AL_{insol} fraction were higher those of AL_{sol} fractions (Table 1). Methoxyl content of AL_{insol} and AL_{sol} fractions decreased with increase of Acid/Ald ratio of alkaline nitrobenzene oxidation products, and that of the AL_{insol} fraction decreased with increase of acidic group. These results suggest together with high Acid/Ald ratio that demethylation (or demethoxylation) and/or introduction of acidic groups occurred during formation of peat.

Conclusions

Peat samples from typical tropical peat swamp around Narathiwat Province, southern Thailand, were collected from various depths of peat profile, and analysed to discuss changes in structural characteristics of lignin in peat samples during peat formation. Cell wall polysaccharides of peat are mineralised at early stage of peat formation. Lignin is significantly modified such as progress of condensation reactions confirmed by alkaline nitrobenzene oxidation, oxidative modification to introduce carboxyl groups by functional group analysis, cleavage of arylglycerol-β-aryl ether intermonomer linkage by analytical ozonation and partially loss of methoxyl groups by methoxyl content determination due to the microbial activities.

Lignin of peat samples collected from different depths was isolated by Björkman's procedure to discuss

its structural feature in detail using spectrometrical procedures (^{1}H-NMR, FTIR and UV spectroscopy) as the first trial in the field of geochemistry and palaeogeography. The signals around 6.6 and 6.9 ppm in ^{1}H-NMR, which are assigned to aromatic protons of syringyl and guaiacyl nuclei, respectively, are considerably weak compared with those of lignin from fresh wood, suggesting the motion of aromatic nuclei is strongly limited by high condensation. The most peaks originating from lignin from fresh wood were also detected in the FTIR spectrum of the peat Björkman lignin. The ionization difference (ΔE_i) spectrum clearly showed the presence of unconjugated and conjugated phenol hydroxyl groups.

Thus, the fragments of lignin in peat modified significantly by microbial activities, become soluble in dilute alkaline solution, but moderately modified fragments of lignin are still insoluble in alkaline. It is expected that further modified fragments of lignin would be soluble in water to colour in brown, and flow-out to ocean through rivers.

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