

ELECTROPHORETIC EVIDENCE OF THE STRUCTURAL SIMILARITY OF DIFFERENT SALT MARSH RELATED HUMIC SUBSTANCES

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Using polyacrylamide gel (PAG) electrophoresis, the humic substances from salt marsh sediment, fresh or dead tissue of *Spartina alterniflora* or from the fungal cultures of *Phaeosphaeria spartinicola* were shown to consist of up to five different molecular fractions. Most of them were of low molecular weight and very mobile in PAG. The distribution of the electrophoretic bands shows almost identical fingerprints of all salt marsh related humic substances tested. This indicates plant and fungal related humic substances to be direct precursors of the sedimentary humic acids.

humic substances; PAG electrophoresis

INTRODUCTION

Salt marshes represent important sources and sinks for a variety of natural and anthropogenic materials in the marine ecosystem. These include refractory carbonaceous substances such as humic and fulvic acids which comprise the major organic components in recent marine sediments (Rashid, 1985). In our previous studies, fresh and standing dead biomass of *Spartina alterniflora* (Loisel.) which is the dominant plant species in salt marshes of the Atlantic and Gulf coasts of the USA, was found to contain appreciable amounts of humic substances (Alberts et al., 1988; Filip et al., 1988). These plant related humic substances showed elemental composition and spectroscopic characteristics in the UV, visible and IR range of light similar to humic and fulvic acids from the salt marsh sediment. In addition, some microscopic fungi epiphytic to *S. alterniflora* were found to produce humic-like melanins with similar characteristics (Filip, Alberts, 1993). To further elucidate whether the *S. alterniflora* and fungal

related humic substances can be considered as direct precursors of the sedimentary humic and fulvic acids, we performed a comparative electrophoretic analysis of these substances.

MATERIALS AND METHODS

Humic substances

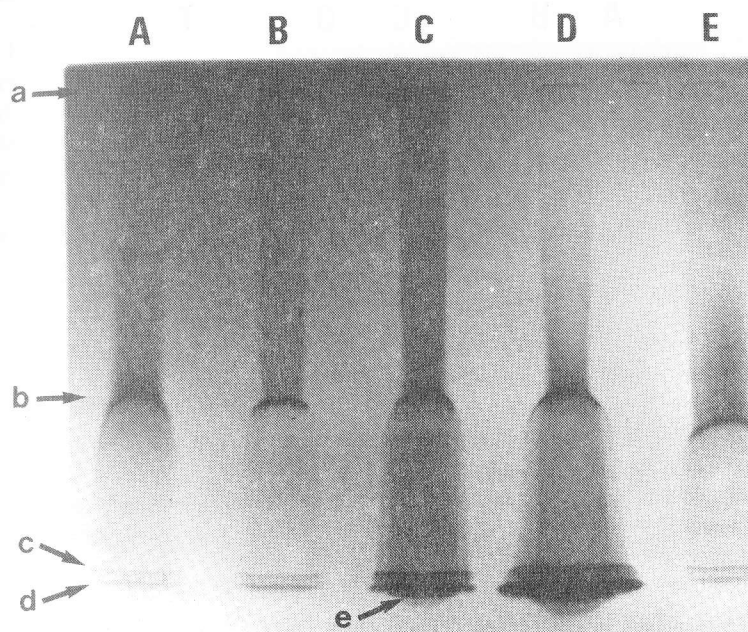
Salt marsh related humic substances were obtained from the fresh and dead plant tissues of *S. alterniflora* or salt marsh sediment of Sapelo Island, Georgia, USA using methods described by Alberts et al. (1988) and Filip et al. (1988). Briefly, dry and finely ground samples were extracted by a mixture of 0.1 M NaOH + 0.1 M Na₄P₂O₇ under N₂, and humic acids were separated from fulvic acids by acidification of the alkaline extract to pH 1.5. Humic acids were then isolated by centrifugation, dialyzed against deionized water and freeze dried. Fulvic acids were concentrated on and eluted from a XAD-resin and also freeze dried. Humic-like melanins were obtained from the cultures of *Phaeosphaeria spartinicola* using similar analytical procedures (Filip, Alberts, 1993). These salt marsh related humics contained between 40–57% C, 2.5–7.6% N, 4.5–7.6% H, and 26–42% O.

Electrophoresis

The electrophoresis was performed in a vertical unit (LKB Sweden) using 10% polyacrylamide gel (PAG). The details of the method were described elsewhere by Trubetskoj et al. (1991). Briefly, freeze dried samples of humic substances were dissolved in Tris borate buffer (pH 8.3) containing 7 M urea, 1% sodium dodecyl sulphate (SDS), 1 mM EDTA and glycerol. Urea and SDS served as denaturing agents to enhance the fractionation of humic substances.

RESULTS

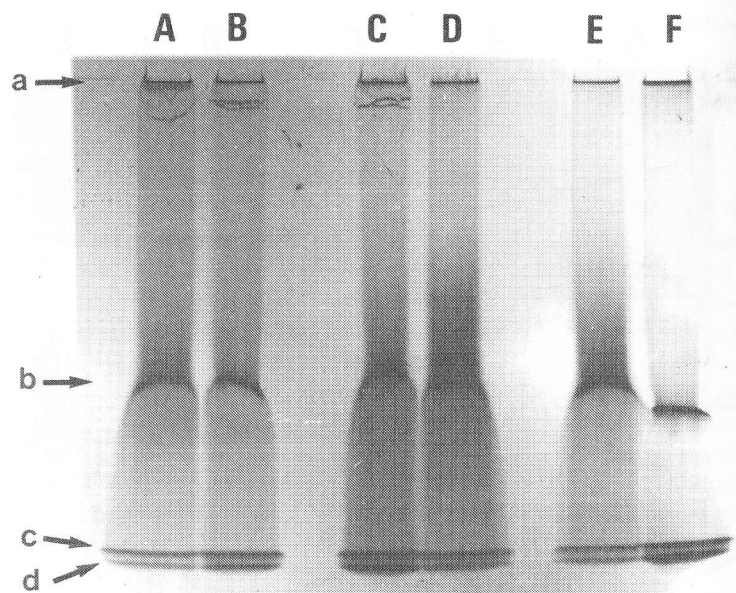
Fig. 1 shows the electrophoregrams of the humic acids extracted from *S. alterniflora* (fresh and dead), humic and fulvic acids from the salt marsh sediment and humic-like melanin from *P. spartinicola*. The sedimentary humic acid were obviously fractionated into five individual bands in the PAG (Fig. 1, C, a-e). The narrow band „a“ which is located at the starting point of the electrophoregram consisted of a humic fraction which did not move in PAG under the electric current used. Four other bands were completely discrete from „a“, indicating the presence of some moderately and highly mobile fractions, all of them dark in color, in the heterogenous mixture of the sedimentary humic acid. The fulvic acid from the salt marsh sediment showed a similar distribution of electrophoretic bands. However, in comparison to the



1. Electrophoregrams in PAG of (A) humic acid from *S. alterniflora*-fresh; (B) humic acids from *S. alterniflora*-dead; (C) humic acid from salt marsh sediment; (D) fulvic acid from salt marsh sediment; (E) fungal melanin from *P. spartinicola* (the symbols a, b, c, d, e for the individual electrophoretic bands)

humic acid the highly mobile fraction „d“ appeared more strongly. The electrophoretic distribution of the individual fractions in *S. alterniflora*-related humic substances represents, in general, a fingerprint of those from the sedimentary humics. This can be recognized from the „a“, „b“, „c“, and „d“ bands. The intensity of these bands is different for the individual preparations, however, their electrophoretic mobility in PAG is the same as that of the sedimentary humic and fulvic acids. The diminished optical intensity of these electrophoretic band was caused by their less intense color.

The electrophoregram of the fungal melanin showed the same number of similarly colored bands as the electrophoregrams of the *S. alterniflora*-related humics. However, the fraction „a“ was much weaker, and the mobility of the fraction „b“ in PAG was somewhat enhanced.



2. Electrophoregrams in PAG of humic acids obtained from *S. alterniflora* decomposed for 10 months in sea water (A) under sterile conditions, (B) inoculated with epiphytic microflora; (C) inoculated with microflora from the salt marsh sediment; (D) inoculated with *Leptosphaeria obiones*; (E) inoculated with *P. spartanicola*; (F) sedimentary humic acid (for a, b, c, d see Fig. 1)

In Fig. 2 the electrophoregram of the sedimentary humic acid was compared with those of *S. alterniflora* (fresh)-related humics which were obtained from the plant decomposed for 10 months in sea water as described in detail by Filip and Alberts (1989). It can be observed that hydrolytic decomposition under sterile conditions (Fig. 2, A) as well as the decomposition mediated by different micro-organisms (Fig. 2, B, C, D, E), all produce organic mixtures with similar electrophoretic distributions of the humic acid fractions. The mobility in PAG of the fraction „b“, however, was somewhat lower, and the intensity of the electrophoretic bands „c“ and „d“ was increased. Humic fraction „e“ became almost undetectable. Humic substances from *S. alterniflora*-dead obtained from the plant material decomposed in sea water (not shown in Fig.) produced electrophoregrams very similar to those demonstrated in Fig. 2.

DISCUSSION

In molecular biology and biochemistry electrophoresis is widely used to identify by fingerprinting nucleic acids and different proteinaceous molecules. In the research on humic substances this fractionation procedure was found useful in the identification of „genetic“ similarities or differences between organic matter from different soil types (Duxbury, 1989). The applicability of electrophoresis to fractionate humic substances was considerably enhanced especially after (1) introducing PAG as the anticonvective electrophoretic medium; (ii) introducing SDS to eliminate charge differences in the electrolyte, and (iii) introducing urea as an disaggregating agent (Castagnola et al., 1978; Klöcking, 1973; Stepanov, Pankhonov, 1969). Application of these principles in a novel methodological modification developed by Trubetskoj et al. (1991), produced homogenous, well reproducible electrophoregrams with a distinct distribution of the individual bands of soil humic substances in PAG. This was demonstrated also in Figs. 1 and 2 for the salt marsh related humic substances. For soil humic acids, Trubetskoj et al. (1992) concluded from the optical density of the eluted electrophoretic fractions a decreasing particle size and/or molecular weight in a sequence from „a“ to „f“ fractions. In their more detailed study Kasparov et al. (1987) reported a particle size of spodosol and molisol humic acids between 2 and 16 nm and molecular weights between 2×10^3 and 2×10^6 daltons in accordance to the mobility (in cm) of the humic preparations in PAG. Because they used more than twice as high PAG concentrations (22.5%), the electrophoregram evaluation made by these authors cannot be directly applied to our electrophoregrams. However, in general (when multiplying the mobility in PAG given by Kasparov et al., 1987, roughly by two), one obtains similar molecular weights and particle sizes for the electrophoretic fractions of humic substances analyzed in our experiment. The „b“ bands would then represent fractions over 104 daltons and of about 4 nm in diameter. The bands „c“, „d“ and „e“ would stand for humic fractions of about 10^3 daltons or less than 2 nm in diameter.

Eloff and Pauli (1976) mentioned in their paper that several other authors stressed the importance of low molecular weight humic substances as agents stimulating plant growth and the overall soil fertility. We assume that low molecular weight humic substances can also affect the copious growth of *S. alterniflora* in salt marshes of the Atlantic and Gulf coast of the USA, where a potential input of 330 kg/ha of fresh *Spartina*-related humic substances may occur annually according to Filip and Alberts (1989). Our electrophoregrams demonstrate clearly the fingerprint similarity between the *Spartina*-related humics and sedimentary humic substances originated from the site where *S. alterniflora* grows. They also indicate that fungi inhabiting *S. alterniflora*, such

as *P. spartanicola*, are capable of producing dark melanins, the molecular fractions of which are very comparable with the humic acids from plant biomass or salt marsh sediment.

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Elektroforetické průkazy strukturní podobnosti různých huminových látek z maršových oblastí.

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Maršové oblasti patří k biologicky nejproduktivnějším přirozeným ekosystémům. Zároveň představují stanoviště stále silněji znečišťovaná antropogenními látkami. Přestože mnohé marše, jako např. na atlantickém pobřeží USA, vykazují monokulturní rostlinný pokryv tvořený travou *Spartina alterniflora*, vyznačují se neobyčejnou ekologickou stabilitou. Jedním z faktorů, které k této stabilitě přispívají, mohou být specifické huminové látky obsažené v pobřežních sedimentech. Tento předpoklad je podložen známými pufracími a sorpčními vlastnostmi huminových látek, avšak i jejich příznivými účinky biologickými. Otázka původu a vlastností maršových huminových látek proto nabývá na významu.

V práci jsou prezentovány výsledky elektroforetického srovnání maršových huminových látek různého původu. Použitím polyakrylamidové gelové elektroforézy (PAGE) byly vyhodnoceny preparáty huminových látek extrahované ze sedimentu, z čerstvé a odumřelé rostlinné biomasy *S. alterniflora*, jakož i látky huminového charakteru vytvořené v dlouhodobých kulturách epifytní plísň *Phaeosphaeria spartanicola*. Většina preparátů se vyznačovala značnou elektroforetickou pohyblivostí, nepochybně související s jejich nízkomolekulárním charakterem. Gelová distribuce jednotlivých frakcí huminových látek byla velmi podobná, což svědčí o úzké strukturní příbuznosti huminových látek získaných z různých zdrojů. Výsledky také naznačují, že produkty epifytních plísň podobné huminovým látkám a stejně tak i látky huminového charakteru obsažené v biomase maršové trávy *S. alterniflora* mohou být považovány za přímé předstupně huminových látek obsažených v pobřežním sedimentu.

huminové látky; PAG elektroforéza

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