



POLYACRYLAMIDE GEL ELECTROPHORESIS OF HUMIC AND FULVIC ACIDS AFTER ACID HYDROLYSIS

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SUMMARY

Two soil humic acids (HA), a marine sediment HA, and a soil fulvic acid (FA), were fractionated by polyacrylamide gel electrophoresis in the presence of denaturing agents before and after 6N HCl hydrolysis. After acid hydrolysis, the intensity of the high molecular size (MS) fraction decreased considerably in all HAs. On the other hand, a new high-MS fraction appeared in the FA after hydrolysis. The electrophoretic data indicate that acid hydrolysis produced a transformation in the humic macromolecule, inducing either depolymerization or condensation, depending on the nature of the humic fraction. Therefore, the advantages and disadvantages of using acid hydrolysis should be carefully considered before this treatment is carried out.

KEYWORDS: Fulvic acids, humic acids, acid hydrolysis, polyacrylamide gel electrophoresis, molecular size distribution.

INTRODUCTION

Humic substances (HS), operationally divided into humic acid (HA, insoluble in acid) and fulvic acid (FA, soluble in acid), exhibit molecular size (MS) heterogeneity [1]. Acid hydrolysis has been considered a method necessary for the study of the core of HS, and is widely used in humus chemistry as a method for HS purification from proteins, polysaccharides and other admixtures considered as non-humic material. In addition, acid treatment (HCl:HF) is used for removal of clays and clay minerals from HA and humins.

Riffaldi and Schnitzer [2] stated that a hydrolytic pretreatment with 6N HCl provides a homogeneous starting material for subsequent analytical and structural investigations. Parsons [3] reported that there are obvious advantages in removing adsorbed or labile-linked materials, which may not be an integral part of the core structure, thereby decreasing the complexities of the degradation products. The labile materials included oligosaccharides, polysaccharides, eptides, proteins, nucleic

acids, phenolic compounds, etc. While 6N HCl hydrolysis led to substantial weight losses (up to 50%), the residues were richer in free radicals, and were possibly more polymerized and more aromatic than the original humic materials [4]. On the other hand, 6N HCl hydrolysis had only minor effect on the distribution of carboxylic and phenolic functional groups in HS of different origin [2, 5, 6]. Moreover, oxidation and pyrolysis products of HS were similar before and after acid hydrolysis [2, 7, 8].

For the study of MS distribution, the methods most frequently used are size exclusion chromatography (SEC) and electrophoresis [9]. Trubetskoj et al. [10] developed polyacrylamide gel electrophoresis (PAGE) in the presence of denaturing agents for fractionation of HS, and showed that the distribution of electrophoretic HA fractions in a gel matrix was based mainly on MS differences [11, 12]. The aim of this work was to apply PAGE for investigating the effects of 6N HCl hydrolysis on the MS distribution of several humic and fulvic acids isolated from different sources.

MATERIALS AND METHODS

Soil humic and fulvic acids used in this study were taken from the A horizons of a Typic Xerorthent (HARK) and a Typic Xerochrept (HAGR and FAGR), soils located in the northern part of the province of Huelva, Spain. Some properties and characteristics of the soils as well as extraction and purification procedures for humic fractions were reported by Saiz-Jimenez et al. [13].

A sample of humic acid extracted from marine sediments (HALH), collected at LaHave Basin on the Scotian Shelf, was also studied [14]. The methods of isolation and purification were described elsewhere [15]. Acid hydrolysis was carried out with 6N HCl in a sealed tube under N₂ for 24 h at 105 °C. The hydrolyzed residue was separated from the supernatant solution by centrifugation, washed thoroughly with distilled water, dialyzed until free of chloride, and lyophilized.

PAGE in the presence of denaturing agents was carried out in 10% polyacrylamide gel according to Trubetskoj et al. [10]. A gel buffer consisting of 89 mM Tris-borate, pH 8.3, with 1 mM EDTA and 7M urea was used; electrode buffer contained 89 mM Tris-borate, pH 8.3, with 1 mM EDTA. For electrophoresis, 0.25 mg of each sample was completely dissolved in 0.05 ml of buffer containing 89 mM Tris-borate, pH 8.3, 7M urea, 1% sodium dodecylsulphate, 0.1 N NaOH, and 1 mM EDTA, and was applied on the gel.

RESULTS AND DISCUSSION

The two soil humic acids of different origin (HARK, HAGR), the marine sediment humic acid (HALH), and the soil fulvic acid (FAGR) were fractionated by PAGE in the presence of denaturing agents before and after 6N HCl hydrolysis (Fig. 1). All untreated HA samples (Fig. 1, a, c, e) were separated into four discrete fractions: A or start zone, which did not move into the gel, and B, C, and D, three narrow, intensely coloured zones. In all soil HAs previously investigated, fractions A corresponded to the fraction excluded in gel chromatography, with $MS < 100,000$ [11, 12], which indicates that this is a real fraction and not an artifact.

Zone B differed greatly from zones C and D in electrophoretic mobility (EM); zones C and D were combined into fraction C+D because of the relatively close electrophoretic behaviour and the difficulties in separating them.

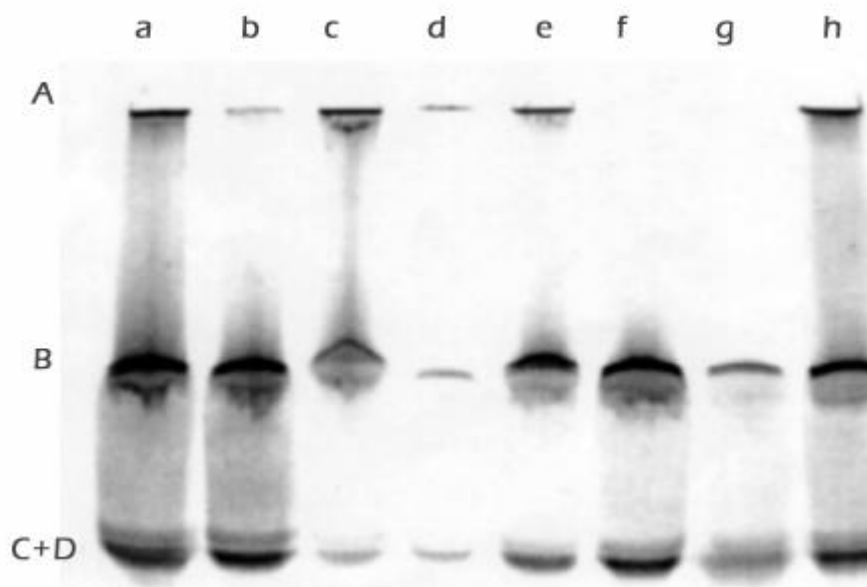
It has been shown previously that MS of electrophoretic fractions decreased with increase of their EM [11, 16]. Fraction A from HAGR had the highest MS, and fraction C+D the smallest MS [12]. Similar results were obtained for the second soil HA and the marine sediment HA.

The soil FA distributed into fractions B and C+D, with only traces of fraction A. This result is in good agreement with data indicating that, on average, the MS of soil FA is somewhat lower than that of HA [1].

After acid hydrolysis, the intensity of fraction A decreased considerably in all HAs (Fig. 1, b, d, f). This could be explained as the result of a degradation (depolymerization and/or release of typical high MS moieties, e.g. proteins) in the whole HA sample. At the same time, some increase in intensity of fractions B and C+D in soil HAs was observed. This could be caused by reduction in MS of the resulting hydrolyzed macromolecule.

Increased intensity in fractions B and C+D in HALH was not observed. Fractions B and C+D of HA samples investigated appeared to be more resistant to acid hydrolysis than was fraction A, probably because protein moieties are poorly represented in these low-EM fractions. This is in agreement with previous studies [17] where the largest amount of amino acids (11.5-21.7%) was found in the high-MS fraction (fraction A), and the smallest (2.1-5.5%) was found in the lowest-MS fraction (fraction C+D).

FIGURE 1
Polyacrylamide gel electrophoresis of HA from Typic Xerorthent before (a) and after (b) 6N HCl hydrolysis, HA from LaHave Basin (c, d), HA from Typic Xerochrept (e, f), and FA from Typic Xerochrept (g, h). A, B, and C+D are discrete electrophoretic zones in the polyacrylamide gel matrix.



Despite the failure of some studies to show significant differences in the distribution of functional groups, after oxidation and pyrolysis of HS before and after acid hydrolysis [5-8], it is evident that the differences in fraction intensities observed in the PAGE were caused by chemical changes of HS occurring during acid hydrolysis.

On the other hand, an intensively coloured fraction A appeared in PAGE of hydrolyzed FAGR (Fig. 1, h), in contrast to the behaviour of HAs. Similar results have been obtained recently for *Epicoccum nigrum* melanin [18]. The appearance of a new A fraction after acid hydrolysis is probably the consequence of a polymerization and/or condensation of the macromolecules and, therefore, an increase in MS.

With regard to fraction A, its decrease in intensity after HA hydrolysis, and its appearance after FA hydrolysis, indicate a different behaviour which is governed by the nature of the humic fraction. Both cases are accompanied by changes in MS distribution. One such behaviour was demonstrated in FAs and fungal melanins and probably corresponded to condensation and/or polymerization processes, as noted by Riffaldi and Schnitzer [2]. A second such behaviour corresponded to HA hydrolysis and release of the protein moieties and, consequently, of hydrolyzable amino acids, together with carbohydrates and some phenols, etc. However, it cannot be ruled out that both behaviours take place in HAs and FAs during acid hydrolysis, although with a different intensity.

It should be noted that for fractions B and C+D, the EM before hydrolysis was identical to that after hydrolysis. This indicates that hydrolyzable humic moieties lost and/or redistributed during hydrolysis of HS seem not to have an essential influence on the electrophoretic behaviour of fractions B and C+D, probably due to the low amount of components prone to acid hydrolysis changes.

CONCLUSIONS

The data shown here indicate that acid hydrolysis produced considerable transformation in the humic macromolecules, inducing either depolymerization or condensation, depending on the nature of the humic fractions. Therefore, the advantages and disadvantages of acid hydrolysis should be carefully considered before this treatment is carried out.

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