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Note

Effect of urea on electrophoretic pattern of soil humic acids

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Numerous systems have been proposed for the fractionation of humic acids[1]. The most effective so far tried is polyacrylamide disc electrophoresis[2]. The greatest difficulty in studying humic acid is the structure of the organic polymer which chelates metallic ions and, through hydrogen bonding, probably interacts both with inorganic colloids and with other organic macromolecules. Therefore an accurate picture of the humic macromolecule can be obtained only if the hydrogen bonds of the aggregates are ruptured.

Klocking[3] has used sodium dodecyl sulphate (SDS) to disaggregate the humic molecule, and has demonstrated its possibilities for the analysis of humic acids. We have now tested a technique used in protein chemistry based on the use of aqueous urea solutions[4] in electrophoretic separation. In urea solutions the electrophoretic pattern of the humic acid is modified giving rise to a separation of subunits which so far have not been unrelated to the soil type.

MATERIALS AND METHODS

Reagents

The humic acid was extracted from one of eight Italian soils selected for structural and pedological correlations[5]. It was prepared by two different treatments, following the method of Kononova[6], either with NaOH (0.1 *M*) or with NaOH (0.1 *M*)–$Na_4P_2O_7$ (0.1 *M*). Electrophoresis was performed using the following products: acrylamide (acryl) and Alcian blau 8GS (Fluka, Buchs, Switzerland), tris(hydroxymethyl)aminomethane (Tris), ammonium peroxydisulphate (persulphate), glycine and urea (Merck, Darmstadt, G.F.R., for analysis), N,N'-methylene-bisacrylamide (bisacryl) (Eastman, Rochester, N.Y., U.S.A.), N,N,N',N'-tetramethyl-1,2-diaminomethane (TEMED) (BDH), Poole, Great Britain).

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Apparatus

A vertical column apparatus (Minivolt, Rome, Italy) was used for all the electrophoretic experiments. Gel polymerization was effected using an Atlas daylight lamp 4300 °K, 15 W.

Procedure

The electrophoresis was performed employing the following solutions:

(a) 48.0 ml of KOH (1.0 *M*) + 4.8 g of glycine + water up to 100 ml.
(b) 1.0 g of acryl + 0.25 g of bisacryl up to 10.0 ml with water.
(b′) 1.0 g of acryl + 0.25 g of bisacryl up to 10.0 ml with 9 *M* urea.
(c) 56.0 mg of persulphate in 10.00 ml of water.
(c′) 17.5 mg of persulphate in 10.0 ml of 10 *M* urea.
(c″) 18.7 mg of persulphate in 10.0 ml of 10 *M* urea.
(d) Saccarose 40% w/v.
(e) 48.0 ml HCl (1.0 *M*) + 36.6 g of Tris + water up to 100 ml.
(f) 38.0 g of acryl + 2.0 g of bisacryl up to 100 ml with water.
(f′) 38.0 g of acryl + 2.0 g of bisacryl up to 100 ml with 0.5 *M* urea.
(f″) 38.0 g of acryl + 2.0 g of bisacryl up to 100 ml with 4.5 *M* urea.
(g) 18.0 mg of persulphate in 10.0 ml of water.
(g′) 18.0 mg of persulphate in 10.0 ml of 5.3 *M* urea.
(g″) 18.0 mg of persulphate in 10.0 ml of 10 *M* urea.
(h) Urea 8 *M*.

The disc-electrophoretic gels were formed in tubes 120 × 6 mm.

The spacer gel was always 0.25 ml × gel, 0.5% w/v polyacryl, pH 10.3, and the separation gel was 2.0 ml × gel, 20% w/v polyacryl, pH 8.9.

The solutions were mixed in the following order:

(1) Standard conditions. Spacer gel: 1.0 ml (a) + 10 μl TEMED + 2.0 ml (b) + 1.0 ml (c) + 2.0 ml water + 2.0 ml (d). Separation gel: 10.0 ml (e) + 25 μl TEMED + 40.0 ml (f) + 30.0 ml (g).

(2) Urea 2.0 *M*. Spacer gel: 1.0 ml (a) + 10 μl TEMED + 2.0 ml (b) + 1.0 ml (c) + 2.0 ml (h) + 2.0 ml (d). Separation gel: 10.0 ml (e) + 25 μl TEMED + 40.0 ml (f) + 30.0 ml (g′).

(3) Urea 4.0 *M*. Spacer gel: 1.0 ml (a) + 10 μl TEMED + 2.0 ml (b) + 3.2 ml (c′) + 1.8 ml (d). Separation gel: 10.0 ml (e) + 25 μl TEMED + 40.0 ml (f′) + 30.0 ml (g″).

(4) Urea 6.0 *M*. Spacer gel: 1.0 ml (a) + 10 μl TEMED + 2.0 ml (b′) + 3.0 ml (c″) + 2.0 ml (d). Separation gel: 10.0 ml (e) + 25 μl TEMED + 40.0 ml (f″) + 30.0 ml (g″).

The samples were prepared by dissolving 4.0 mg of humic acid in: (1) standard conditions, 0.8 ml of NaOH (0.1 *M*); (2) urea 2.0 *M*, 0.6 ml of NaOH (0.1 *M*) + 0.2 ml of 8.0 *M* urea; (3) urea 4.0 *M*, 0.48 ml of NaOH (0.1 *M*) + 0.32 ml of 10.0 *M* urea; (4) urea 6.0 *M*, 0.32 ml of NaOH (0.1 *M*) + 0.48 ml of 10.0 *M* urea. To all the samples 0.2 ml of glycerine were added.

The volume applied to the gel was always 0.100 ml. The electrophoretic buffer was Tris–glycine pH 8.3 (0.6 g of Tris + 2.88 g of glycine, made up to 1.0 l with water). The polarity was negative at the top and positive at the bottom.

For the first 5 min of each electrophoretic run, the voltage remained at 100 V

(*ca.* 1.5 mA × gel), then was increased to 200 V (*ca.* 3.0 mA × gel) until separation was complete. No markers were used, because the humic acid was already coloured and even the fast marker chosen was always slower than the front band.

The fixing solution was 10% lead acetate in 10% acetic acid, and the fixing time a minimum of 6 h. The staining solution was 0.2% Alcianblau 8GS in 7.5% acetic acid, and the staining time 6 h. Destaining was performed under stirring for at least 24 h.

RESULTS AND DISCUSSION

The electrophoretic pattern of the humic acid shows, in normal conditions, three principal components (Figs. 1 and 2). For gel A, fraction *a* at the top of the gel probably consists of high molecular weight or highly hydrogen-bonded components; fraction *c* is the fast one, with a high charge and small dimensions; the middle fraction, *b*, has the highest concentration. The same electrophoretic pattern was found for the humic acids of the eight different soils examined.

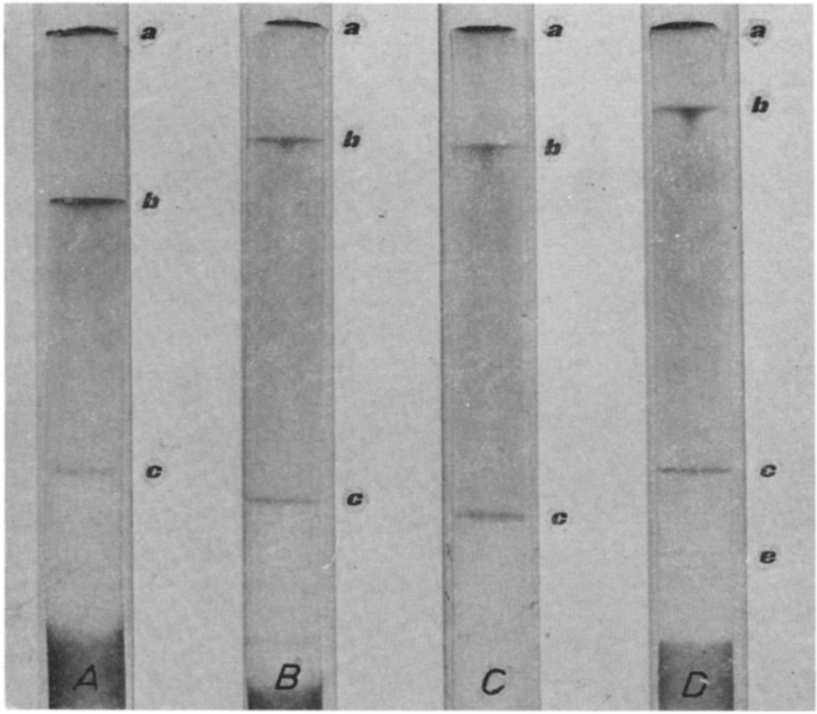

Fig. 1. Polyacrylamide disc gel electrophoresis of humic acid obtained by extraction of the soil sample with NaOH with increasing urea concentration. A, 0.0 *M* urea; B, 2.0 *M* urea; C, 4.0 *M* urea; D, 6.0 *M* urea.

These results suggest that all soil humic acids are not statistical polymers, but that they contain constant and uniform molecular fractions, thus enabling gross information, to be obtained from structural studies for all humic acids. On the other

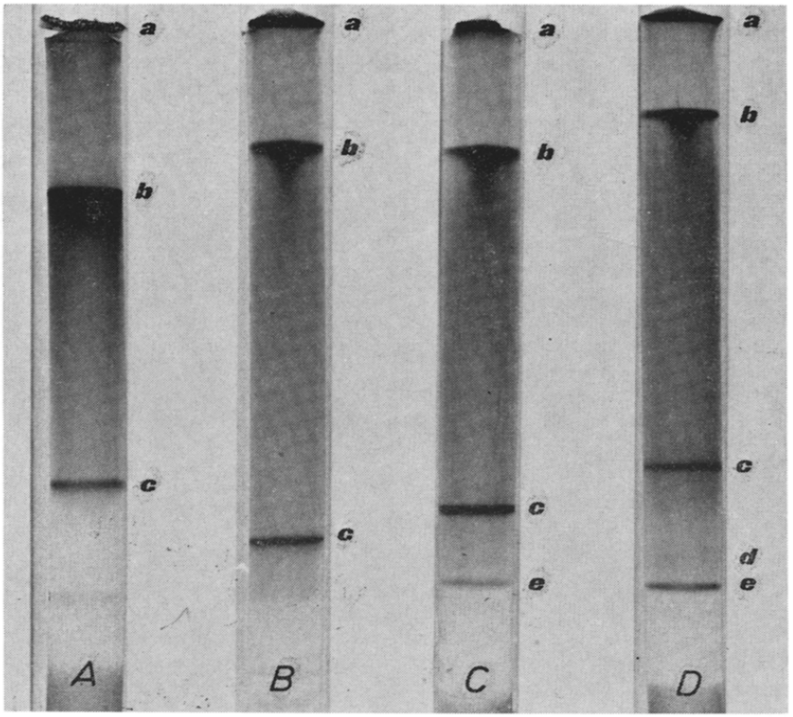

Fig. 2. Polyacrylamide disc gel electrophoresis of humic acid obtained by extraction of the soil sample with NaOH–$Na_4P_2O_7$ with increasing urea concentration. A, 0.0 *M* urea; B, 2.0 *M* urea; C, 4.0 *M* urea; D, 6.0 *M* urea.

hand, it is not possible to use humic acid as a characteristic acid component of the soil.

The differences between Figs. 1 and 2 are due to the different extraction solutions used. Use of NaOH consistently gave a higher ash content than NaOH–$Na_4P_2O_7$. The low solubility of the humic acid in NaOH prevented a proper comparison with the pyrophosphate extraction.

The use of urea in different concentrations as disaggregant shows remarkable effects on bands *b* and *c*, but not in band *a*. Increase of the urea concentration causes a decrease in the *b* band intensity and a proportional increase in the *c* band intensity. Two new bands appear (*d* and *e*); band *d* is always faint whatever the urea concentration. Band *e* is sharp and moves with the electrophoretic front; with 6.0 *M* urea a further disaggregation of the *c* band appears towards the *e* band.

The following conclusions were drawn: (1) band *a* is a strongly linked macropolymer; (2) band *b* is a uniform polymer made of specific subunits, corresponding to band *c*; (3) band *c* consists of small, "quick" subunits (see band *e*, 6.0 *M* urea) running at least as fast as the front, which prevented us from establishing whether the band consists of only one or more components so small that the gel cannot sieve them; (4) in addition there are faint bands probably belonging to band *b*; and (5) a colour diffusion was noted from band *b*, probably indicating the presence in the humic acid of statistical components.

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