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Short Communication

Biofertilizers: exceptional calcium binding affinity of a sheath proteoglycan from the blue-green soil alga *Nostoc calcicola*

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Blue-green algae (also called cyanobacteria) have an impressive ability to colonize infertile substrates such as volcanic ash (Campbell, 1909; Brock, 1973) and desert sand and rocks (Friedmann & Galun, 1974; Metting, 1991; Painter, 1993). Growth without exogenous combined nitrogen is possible because heterocystous, filamentous genera are autonomous (non-symbiotic) nitrogen-fixers (Bothe, 1982). The ability to derive phosphate and micro-nutrients from insoluble minerals is, however, understood only in outline.

In fertile soils, mineral diagenesis, with release of plant nutrients in soluble form, is brought about by humic acids, produced by the decay of higher plant material (Kononova, 1961). The mechanism entails the sequestration of multivalent metal cations such as Ca^{2+}, Al^{3+} and Fe^{3+} (Schnitzer, 1978). Since the algae will grow on volcanic ash, which contains no organic matter, it must be presumed either that the living cells synthesise a substance that functions in the same way, or that it is formed upon the death and decay of the algal trichomes (Verma & Martin, 1976; Zunino & Martin, 1977).

Several indirect observations support the former inference, especially with regard to the sequestration of calcium. Most filamentous species require abundant Ca^{2+} for growth (Guillard & Lorenzen, 1972), and some will even bore their way into limestone, to form colonies inside the rock (Whitton & Potts, 1982). The title species is one of these, as its name, *calcicola* (chalk-inhabiting) implies. The 'survival value' of this activity relates not only to nutrition, but also the fact that excessive solar radiation inhibits growth. In littoral mats (stromatolites), the gelatinous sheaths of such species are often observed to contain microcrystals of calcite, which protect the cells from excessive light (Van Liere & Walsby, 1982). Such mineralisation may develop into incrustation, and ultimately into petrification (Schopf & Walter, 1982).

The optimum pH for growth of blue-green algae is typically in the range of 7·5–9·5, and few species will grow significantly below pH 6. The boring into, dissolution, and subsequent redeposition of calcium carbonate may be controlled by changes in pH, but these could scarcely extend to below pH 7 during active growth, when the boring occurs, and when the pH tends to rise because of the uptake of bicarbonate by cells. It is, therefore, hard to explain these phenomena without assuming the participation of an endogenous bio-chelator, synthesised by healthy cells.

Since the exocellular (sheath) proteoglycans are already known to be responsible for the adhesion of the algal trichomes to solid surfaces (Flaibani *et al.*, 1989; Painter, 1993), it is logical to expect that they would also incorporate the sequestering function. Definitive evidence is now reported that this is the case, and that both the glycan and polypeptide moieties participate in the binding mechanism.

Nostoc calcicola Geitler, strain 79WA01 is sold commercially as a biofertilizer in Washington State, USA, and is currently being evaluated for possible use in desert reclamation (Reynaud & Metting, 1988; Flaibani *et al.*, 1989; Knutsen & Metting, 1991; Painter, 1993). Details of its laboratory cultivation, the isolation of sheath proteoglycan fractions, and their analysis for sugars and amino-acids, have been reported (Flaibani *et al.*, 1989). Cation binding capacities and selectivity

coefficients, K_{Mg}^{Ca}, defining the affinity for Ca^{2+} relative to that for a reference cation of the same valency (Mg^{2+}), were measured as in a cognate study of peat humic acids (Smidsrød & Painter, 1984). Each test sample had the same cation-binding capacity (expressed in mEq/g) for Na^+, Mg^{2+} and Ca^{2+}, which also agreed with the equivalent weight calculated from the sugar and amino-acid analyses (Table 1). There was, therefore, no site-specific binding of the type recently reported for some anionic chitin derivatives (Uraki *et al.*, 1993).

Figure 1 shows Scatchard plots of K_{Mg}^{Ca} against X_{Ca}, the fraction of the total number of binding sites in the proteoglycan that are occupied by Ca^{2+}. Results are shown for three separate proteoglycan fractions that differ in the way in which their anionic groups are distributed between glycuronoglycan and polypeptide moieties (Table 1). These figures were calculated from the total hexuronic acid contents (Gal_pA and Glc_pA) of the former, and from the total contents of non-amidated Asp and Glu in the latter (Flaibani *et al.*, 1989; Painter, 1993). For comparison, results for a sample of humic acid isolated from peat-bog water (Smidsrød & Painter, 1984) are included.

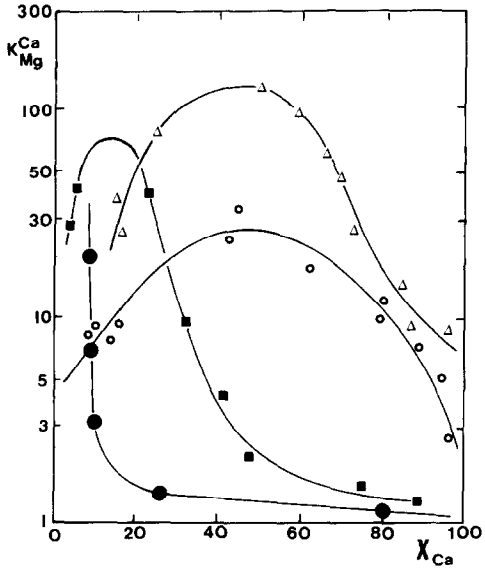

Fig. 1. Semi-logarithmic plots of calcium-binding selectivity (K_{Mg}^{Ca}) against the percentage (X_{Ca}) of binding sites occupied by Ca^{2+}. ●: PG_{edta}^{500}; ■: PG_{cfc}^{20}; △: PG_{hw}^{500}; ○: humic acid.

The value of $(K_{Mg}^{Ca})_{max} = 130$ obtained for PG_{hw}^{500} is four times higher than that for the peat humic acid, and is the highest selectivity for Ca^{2+} ever recorded for any biopolymer in this laboratory. The values of X_{Ca} corresponding to $(K_{Mg}^{Ca})_{max}$ roughly reflect the proportions of anionic groups in the polypeptide moieties alone, and suggest a dominating role for these binding sites. The glycuronoglycan moieties must also contribute to the selectivity, however, because fairly high K_{Mg}^{Ca} values persist when X_{Ca} exceeds the capacity of the binding sites in the polypeptide moiety alone. This is especially evident with PG_{hw}^{500}, in which all of the binding sites are associated with significant selectivity. This argument is explained in greater detail in a paper on humic acids from peat (Smidsrød & Painter, 1984).

Whereas the magnesium salts of the proteoglycans form viscous solutions in water, these are converted into voluminous gels by Ca^{2+}, even at low values of X_{Ca}. This fact, together with the positive, initial slopes of the Scatchard plots for PG_{cfc}^{20} and PG_{hw}^{500}, suggests that high selectivity is associated with strongly co-operative cross-linking by Ca^{2+}. The cross-linkages must be chelate bridges, and a significant proportion of them must be of the 'mixed-ligand' type, with a hexuronic acid residue as one ligand, and an amino-acid sequence containing Asp or Glu as the other.

Not surprisingly, it is hard to remove calcium from these complexes under mild (non-acidic) conditions. Repeated dialysis against a large excess of EDTA is required. Any lack of thoroughness in this operation results in a product that contains firmly bound EDTA as well as calcium. This seems to confirm the formation of chelate bridges of the 'mixed-ligand' type.

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Table 1. Distribution of anionic groups in proteoglycan fractions

Fraction	N (%) × 6·25	Cation-exchange capacity (mEq/gram)		
		Glycan[a] (G)	Protein[b] (P)	$100P/(P+G)$
PG_{edta}^{500}	3·4	1·0	0·056	5·3
PG_{cfc}^{20}	7·9	0·74	0·13	15
PG_{hw}^{500}	26	0·42	0·45	52
Humic acid	16	0·54	0·92[c]	63

[a] Gal_pA + Glc_pA; [b] Asp + Glu − NH_3; [c] non-carbohydrate moiety comprises polypeptide (28%) and a dark brown, partly aromatic chromophore (72%).

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