Biochimica et Biophysica Acta, 600 (1980) 205—211

BBA 78814

BACTERIA-MEDIATED UPTAKE OF CHOLINE SULFATE BY PLANTS

BACTERIAL EFFECTIVENESS

KARI G. BAKKERUD and PER NISSEN *

Department of Microbiology and Plant Physiology, University of Bergen, N-5014 Bergen (Norway)

(Received October 8th, 1979)

Key words: Choline sulfate; Bacteria-mediated uptake; Permease induction; (Pseudomonas tolaasii, Hordeum vulgare L.)

Summary

The ability of bacteria to cause rapid uptake of choline sulfate in plants, i.e., effectiveness, was studied using *Pseudomonas tolaasii* and excised roots of barley (*Hordeum vulgare* L.). Once effective, bacteria remained so after being killed by treatments which cause little damage to their outer structure. However, effectiveness was destroyed by disruption of the cell wall, protein reagents, a mild heat treatment or removal of Mg^{2+}. Effective bacteria adsorbed choline sulfate. This adsorption had characteristics similar to those of bacterial effectiveness (magnesium requirement, high substrate specificity). These results indicate that a proteinaceous structure on the bacterial surface binds and, somehow, transfers choline sulfate to the plant.

Introduction

Certain Gram-negative bacteria prevalent around plant roots can mediate uptake of choline sulfate by plants [1—3]. Three induction processes occurring in sequence are involved in this complex and, as yet, poorly understood interaction [2,3]. The first process is substrate induction of a bacterial permease for choline sulfate. The bacteria can then acquire the ability to mediate plant uptake of this sulfate ester, i.e., effectiveness, in a second induction process. Effective bacteria in contact with plant roots will cause rapid uptake of choline sulfate in the plant. Removal of the bacteria results in loss of induced

* To whom correspondence should be addressed.

plant uptake unless the plants have completed the third and final induction process. After completion of this process, the bacteria can be removed with no loss in the rate of uptake.

We report here how various treatments of effective bacteria affect their ability to (i) mediate plant uptake of choline sulfate and (ii) adsorb this organic zwitterion. The results indicate that bacterial effectiveness requires the formation of a binding protein for choline sulfate on the bacterial surface.

Materials and Methods

Bacteria (*Pseudomonas tolaasii* PT 109, now provisionally [4] assigned to *Pseudomonas fluorescens* biotype II) and seedlings of barley (*Hordeum vulgare* L. cv. Herta) were grown essentially as previously described [1,3]. Bacteria grown for 1 day with $5.5 \cdot 10^{-3}$ M choline sulfate as the source of sulfur were made effective by shaking for 20 h at room temperature in solutions of 1 10^{-3} M choline sulfate plus $1 \cdot 10^{-2}$ M $MgCl_2$. Bacteria were assayed for effectiveness by their ability to mediate uptake of choline [^{35}S]sulfate ($1 \cdot 10^{-5}$ M) by excised roots from 7-day-old barley seedlings (30 min uptake at room temperature, 10 min wash in cold water), (cf. also Refs. 1 and 3). Adsorption of choline [^{35}S]sulfate ($1 \cdot 10^{-5}$ M) to bacteria was assayed by filtering 1.0 ml aliquots of the bacterial suspensions ($A_{580} = 0.25$) through 0.45 μm membrane filters, washing with 10 ml of water (three times) and determining the radioactivity of the dried filters by liquid scintillation counting.

Choline sulfate (unlabeled and ^{35}S-labeled) and 2-dimethylaminoethyl sulfate were synthesized by methods referred to previously [1,5].

Results

Bacteria-mediated uptake in plants

Contrary to previous indications [3], effective bacteria can be killed without losing their ability to mediate uptake of choline sulfate in the plant. Fig. 1 shows that the bacteria were killed within 10 s by an ultraviolet treatment while still retaining about 70% of their effectiveness. It took 2 min to halve and 30 min to completely destroy bacterial effectiveness. To determine whether the bacteria were indeed killed or whether they were only prevented from dividing, bacteria were irradiated for 5 or 10 min before or after attempts to make them effective had been made.

Irradiated bacteria could not subsequently be made effective, indicating that they were killed by the ultraviolet treatment. Bacteria irradiated after being made effective retained some effectiveness, 54 and 26% for the 5- and 10-min treatments, respectively, in agreement with Fig. 1.

The differential effect of ultraviolet irradiation on bacterial death and effectiveness could be apparent rather than real if the number of bacteria greatly exceeded that needed for maximal induced plant uptake. However, this was not the case. Induced plant uptake of choline sulfate was roughly proportional to the number of bacteria for bacterial concentrations similar to those used in these experiments (cf. also Ref. 3).

Other treatments, which also cause little damage to the bacterial surface,

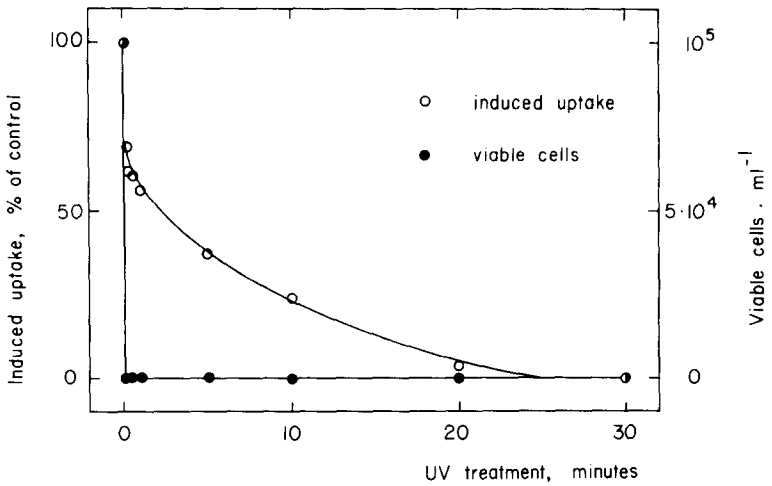

Fig. 1. Differential effect of ultraviolet irradiation on bacterial effectiveness and viability. Suspensions (A_{580} = 0.46, depth 3 mm) of effective bacteria were placed 20 cm below ultraviolet tubes (Philips 57415 P/40 A6, 15 W) for indicated periods and assayed for effectiveness and viability.

similarly killed all or most of the bacteria with little or no loss of effectiveness. Lucas [6] found that *Pseudomonas aeruginosa* collected by suction on a membrane filter will rupture when washed with water. A similar osmotic shock also opened cells of *P. tolaasii*. Electron microscopy indicated that the rupture was mainly in one end and that most of the surface remained intact, as did the ability to mediate plant uptake of choline sulfate. Decompression in a nitrogen atmosphere (decompression bomb, Artisan Industries Inc., Waltham, MA) also ruptured most of the bacteria as indicated by a marked increase in viscosity and by plate counts. However, bacterial effectiveness was retained, presumably due to the mild conditions of disintegration (inert atmosphere, no heat production).

Effectiveness was destroyed by sonication or by lysing the cells with lysozyme and EDTA [3]. Extrusion of the bacteria through a small orifice (French press) also destroyed effectiveness, presumably because of severe disruption. Spheroplasts produced from effective bacteria by treatment with a mixture of Tris, sucrose and lysozyme [7] were ineffective.

Effectiveness was also destroyed by treatment of bacteria with low concentrations of various SH- and protein reagents (Table I). 4-Diazobenzenesulfonic acid, which does not penetrate membranes, was also inhibitory ($I_{50} \simeq 5 \cdot 10^{-4}$ M), as were molar concentrations of urea and guanidine hydrochloride. However, high concentrations of pronase (*Streptomyces griseus* protease, Calbiochem) were without effect. This is in agreement with previous experiments [3] in which a protease, as well as a variety of other enzymes, were without effect. The detergents, dodecyl sulfate and deoxycholate, were only slightly inhibitory.

Effectiveness was reduced by at least two-thirds by heating a suspension of effective bacteria to 45°C for 1 min or less, also in agreement with previous results [3]. The effectiveness was destroyed almost completely by heating to

TABLE I

EFFECT OF SH- AND PROTEIN REAGENTS ON BACTERIAL EFFECTIVENESS

Effective bacteria were treated with reagents for 30 min at room temperature, washed twice and assayed for effectiveness. Control: bacteria treated with water.

Reagent (M)	Bacteria-mediated uptake in roots (% of control)
N-Ethylmaleimide ($1 \cdot 10^{-5}$)	7
N-Ethylmaleimide ($1 \cdot 10^{-4}$)	0
$HgCl_2$ ($1 \cdot 10^{-6}$)	48
$HgCl_2$ ($1 \cdot 10^{-5}$)	0
2,4-Dinitrofluorobenzene ($1 \cdot 10^{-5}$)	34
2,4-Dinitrofluorobenzene ($1 \cdot 10^{-4}$)	10
Trichloroacetic acid ($1 \cdot 10^{-5}$)	112
Trichloroacetic acid ($1 \cdot 10^{-4}$)	0

50°C. This heat lability remained the same whether or not the bacteria were previously killed by osmotic shock. Addition of Ca^{2+} or Mg^{2+} ($1 \cdot 10^{-2}$ M) had a protective effect, about 30% effectiveness remaining after heating to 50°C.

In contrast to the latter result, the structure conferring effectiveness was found to specifically require Mg^{2+} (Table II). Treatment of bacteria with EDTA severely reduced their effectiveness. This reduction could be at least partly counteracted by subsequent addition of Mg^{2+}. However, addition of Ca^{2+} destroyed all or most of whatever effectiveness remained after the EDTA treatment. Ca^{2+} probably acts by removing any remaining Mg^{2+} by exchange, as indicated by the finding that an excess of Ca^{2+} prevented any restoration by Mg^{2+}.

Adsorption of choline sulfate to effective bacteria

Effective bacteria can also adsorb choline sulfate. Fig. 2 shows that adsorption to effective bacteria occurred rapidly, while there was little or no adsorption to bacteria that had not been made effective, or to effective bacteria made ineffective by a prolonged ultraviolet treatment. The bacteria were ruptured after collection on membrane filters and the radioactivity therefore reflects adsorption rather than absorption of choline sulfate. Furthermore, the radio-

TABLE II

REQUIREMENT OF Mg^{2+} FOR BACTERIAL EFFECTIVENESS

Effective bacteria were treated with EDTA for 10 min at room temperature, washed, similarly treated with water, Mg^{2+}, Ca^{2+}, or Mg^{2+} plus Ca^{2+}, washed and assayed for effectiveness. Control: bacteria treated with water. EDTA present at 0.1 M.

Treatment	Bacteria-mediated uptake in roots (% of control)
EDTA + H_2O	12
EDTA + $2 \cdot 10^{-2}$ M $MgCl_2$	49
EDTA + $2 \cdot 10^{-2}$ M $CaCl_2$	2
EDTA + ($2 \cdot 10^{-2}$ M $MgCl_2$ + $2 \cdot 10^{-1}$ M $CaCl_2$)	1

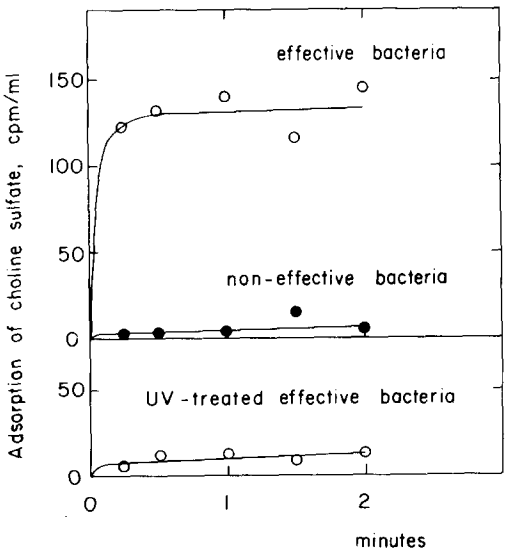

Fig. 2. Time course of adsorption of choline sulfate to bacteria. Effective bacteria, non-effective bacteria (only induction process I completed) and effective bacteria made ineffective by ultraviolet irradiation for 20 min (cf. Fig. 1) were assayed for ability to adsorb choline sulfate.

activity could be rapidly exchanged by washing with an excess of unlabeled choline sulfate.

Mg^{2+} is not only required for bacterial effectiveness but also for adsorption. In an experiment similar to that in Table II, treatment with EDTA decreased adsorption of choline sulfate to 62% of the control. Addition of Mg^{2+} restored adsorption to 91%, while Ca^{2+} decreased it still further to 49%.

Adsorption of choline sulfate shows a high degree of specificity. A 100-fold excess of the close analog, 2-dimethylaminoethyl sulfate, resulted in little or no inhibition (Table III). As expected, unlabeled choline sulfate decreased adsorption of the labeled compound, while Na_2SO_4 had no effect. These results, as well as the unexplained stimulation by choline chloride, are similar to previous results for bacterial absorption of choline sulfate and for bacteria-mediated plant uptake [3]. In contrast, constitutive uptake of choline sulfate in plants is much less specific [5].

TABLE III

SPECIFICITY OF ADSORPTION TO EFFECTIVE BACTERIA

Adsorption to effective bacteria was assayed after incubation in indicated solutions for 30 s at room temperature (cf. Fig. 2). Choline sulfate present at $1 \cdot 10^{-5}$ M; all additions present at $1 \cdot 10^{-3}$ M.

Solution	Adsorption (% of control)
Choline [^{35}S]sulfate	100
+ dimethylaminoethyl sulfate	95
+ choline sulfate	38
+ Na_2SO_4	109
+ choline chloride	200

Discussion

The results in this communication, taken together with the requirement for contact between bacteria and plants [3], strongly indicate that a proteinaceous structure on the bacterial surface is involved in bacteria-mediated uptake of choline sulfate by plants. The structure is formed while bacteria are being made effective in the second induction process, it is heat-labile and requires Mg^{2+}. There are probably steric reasons why proteolytic enzymes, in contrast to low-molecular weight protein reagents, have no effect on the structure. The requirement for Mg^{2+} explains why bacteria cannot become effective when grown in the presence of high concentrations of citrate or other ions [3]. Upon addition of high concentrations of Mg^{2+}, the bacteria did become slightly effective in a growth medium containing choline sulfate.

Ca^{2+} can readily exchange with Mg^{2+}, but its substitution for Mg^{2+} renders the structure ineffective. In a possibly analogous situation, Mg^{2+} — but not Ca^{2+} — stabilizes the low-temperature form of a heat-modifiable protein in the outer membrane of *Escherichia coli* [8].

Adsorption of choline sulfate to the proteinaceous structure is required for bacterial effectiveness (Fig. 2). Upon contact between bacteria and plants, the bound choline sulfate is then apparently transferred to the plant. The suggestion that the effective structure on the bacterial surface functions as a binding protein for the plant is supported by the finding that the substrate specificity for induced plant uptake is indistinguishable from that of bacterial adsorption. Transport of choline sulfate from the surface of the root through the mucilaginous layer and the cell wall to the plasmalemma of the cortical cells may be by diffusion or may be mediated in some unknown manner. Transport through the plasmalemma may possibly be mediated by one of the plant's constitutive mechanisms for uptake of choline sulfate [5].

It has not been possible to obtain effective subcellular entities or fragments by differential centrifugation of intact effective bacteria or effective bacteria ruptured by various physical methods. It may be that effectiveness is dependent on the bacterial surface remaining at least partially intact. Supernatants, filtrates, etc., from effective bacteria are also ineffective [3]. No difference between non-effective (induction process I completed) and effective (process II completed) bacteria has been found upon transmission or scanning electron microscopy of intact, sectioned or freeze-etched bacteria.

The mechanism by which the plant in the final induction process acquires the ability to take up choline sulfate rapidly in the absence of bacteria remains unknown. This process requires the presence of effective bacteria; that is, bacteria with adsorbed choline sulfate, and probably plant protein synthesis [3]. The process can occur in a variety of plants and tissues [3] and leads to the formation in the plant of an uptake mechanism with specificity identical to that of the bacterial permease and the binding protein on the bacterial surface. A transfer of information in some form or other from the bacteria to the plant [1—3] therefore remains a distinct possibility.

Acknowledgments

Some of this work was performed in the Department of Biology, University of Calgary. We thank Professor J.W. Costerton and his co-workers for their interest and help, Dr. H. Breteler for helpful comments on the manuscript and the Norwegian Research Council for Science and the Humanities for financial support.

References

1 Nissen, P. (1968) Biochem. Biophys. Res. Commun. 32, 696—703
2 Nissen, P. (1971) in Informative Molecules in Biological Systems (Ledoux, L.G.H., ed.), pp. 201—212, North-Holland, Amsterdam
3 Nissen, P. (1973) Sci. Rep. Agric. Univ. Norway 52 (20), 1—53
4 Buchanan, R.E. and Gibbons, N.E. (1974) Bergey's Manual of Determinative Bacteriology, 8th edn., p. 223, Williams and Wilkins, Baltimore
5 Nissen, P. (1974) Physiol. Plant. 30, 307—316
6 Lucas, J.J. (1972) Choline Sulfatase in Microorganisms. Ph.D thesis, University of California, Davis, (Diss. Abstr. B (1973) 34, 522)
7 Fitzgerald, J.W. and Laslie, W.W. (1975) Can. J. Microbiol. 21, 59—68
8 McMichael, J.C. and Ou, J.T. (1977) J. Bacteriol. 132, 314—320