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THE INHIBITION OF SALT UPTAKE BY D-SERINE

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Abstract—The uptake of potassium, sodium, chloride, nitrate, phosphate, and sulphate ions by washed slices of red beetroot tissue is inhibited by D-serine; 1–5 mM D-serine reduces salt uptake by 40–50 per cent in 3 hr at 25°. D-Serine also inhibits sodium, phosphate, and sulphate uptake by washed carrot and turnip slices and by excised pea roots. D-Alanine and D-threonine also inhibit potassium and sulphate uptake by beet slices but are not as effective as D-serine, whereas L-serine, L-alanine, and L-threonine show no effect. Pre-treatment with D-serine inhibits the subsequent uptake of salt from a solution lacking the inhibitor. The uptake of D-serine is not affected by a variety of potassium and sodium salts. D-Serine does not affect the efflux of accumulated sulphate. The rate of oxygen uptake by beet slices and pea roots in the presence of salt is not affected by D-serine nor is the lower rate of oxygen uptake in water. D-Serine appears to uncouple salt uptake from respiration.

INTRODUCTION

THE mechanism of salt uptake by plants is not known but experiments with inhibitors such as cyanide and carbon monoxide have confirmed that it is dependent on respiration, at least in non-green tissues and some of the current ideas of the mechanism of salt uptake suggest a close connexion between the two.[1,2] The cornerstone of these theories is the phenomenon of salt respiration, that is, the increase in the rate of oxygen uptake displayed by some plant tissues when they are transferred from water to a salt solution; great importance has been attached to this phenomenon as possibly indicating the bringing into play of some respiratory component particularly involved in the process of salt accumulation. However, it is also true that salt respiration cannot always be demonstrated when a tissue is taking up salt,[3] and alternative explanations for salt respiration when it does occur are not hard to devise. It is not surprising that compounds which inhibit or uncouple respiration also inhibit salt uptake; a more useful compound for the understanding of the process of salt uptake would be one which had no discernible effect on respiration but which yet inhibited the uptake of salt. Sutcliffe recently reported[4] that the antibiotic chloramphenicol when supplied to plant tissues

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[1] R. N. ROBERTSON, *Biol. Rev.* **35**, 231 (1960).
[2] G. E. BRIGGS, A. B. HOPE and R. N. ROBERTSON, *Electrolytes and Plant cells*, Blackwell Scientific Publications, Oxford (1961).
[3] R. HANDLEY and R. OVERSTREET, *Science* **135**, 731 (1962).
[4] J. F. SUTCLIFFE, *Nature, Lond.* **188**, 294 (1960).

at high concentrations would inhibit the uptake of ions without affecting the uptake of oxygen, and commented that this finding supported the suggestion of Steward and Millar[5] that salt uptake was more closely connected to protein synthesis than to respiration *per se*.

Previous work[6] has shown that the D-isomer of the amino acid serine inhibits the uptake of sulphate by beet slices; the present paper extends this finding.[7] The effect of D-serine and some structurally related compounds on the uptake of five other ions besides sulphate is reported, and three plant tissues besides beet slices have been used; some of the ways in which D-serine could be exerting its effect have been eliminated.

RESULTS

1. *Inhibition of Ion Uptake by* D-*Serine in Several Tissues*

Many of the experiments on the inhibition of salt uptake by D-serine have been carried out with sulphate. The uptake of sulphate is reduced to about half by 1–5 mM D-serine in 3 hr (Fig. 1) and therefore succeeding experiments were performed with these conditions. The effect is not restricted to sulphate; D-serine reduces the uptake by plant tissues of at least five other ions, including both cations and anions. The inhibition of the uptake of sodium, phosphate and potassium ions by beet and carrot slices is shown in Table 1. Similar inhibitions have been obtained of the uptake of nitrate and chloride by beet slices and of the uptake of sulphate by turnip slices and excised pea roots (Table 4).

Table 1. Effect of D-serine and chloramphenicol on sodium, phosphate and potassium uptake.
Samples of 30 three-day-washed tissue slices were shaken in 4 ml NaH_2PO_4 (5 mM) or KCl (2 mM) with and without the inhibitors for 5 hr at 25°. The uptake of ions was determined as described in the text. The figures are averages derived from triplicate flasks

	(μ equiv./g fresh weight)				
	Uptake of sodium		Uptake of phosphate		Uptake of potassium
Compounds added	Beet	Carrot	Beet	Carrot	Beet
None	10·3	7·4	8·1	5·9	10·1
D-Serine (1 mM)	5·6	6·1	3·4	5·2	4·9
D-Serine (5 mM)	3·4	5·2	1·8	3·9	3·7
Chloramphenicol (3·1 mM)	3·4	5·4	0·8	4·1	3·4

Uptake of D-serine by the tissue is necessary for a demonstration of the inhibitory effect on salt uptake, and inhibition appears to depend to some extent on the amount of D-serine taken up. From Table 2 the effect of various concentrations of D-serine on sulphate uptake can be seen. In the range 1–5 mM the rate of uptake of D-serine is approaching saturation; increasing the concentration from 1–5 mM increases the uptake by only about two-fold. When the concentration of D-serine is increased to 50 mM only a relatively small further increase in the degree of inhibition of salt uptake is obtained. With potato slices no uptake of D-serine was found, and no inhibition of salt uptake could be demonstrated.

[5] F. C. Steward and F. K. Millar, *Symp. Soc. Exp. Biol.* **8**, 367 (1954).
[6] R. J. Ellis, *Phytochemistry* **2**, 129 (1963).
[7] R. J. Ellis, K. W. Joy and J. F. Sutcliffe, *Biochem. J.* **87**, 39P (1963).

2. *Effect of Related* D- *and* L-*Amino Acids*

The D-isomers of alanine and threonine, the two amino acids most closely related in structure to serine, also inhibit the uptake of sulphate (Table 2). However, D-serine is more effective than either D-alanine or D-threonine and this is especially evident when the uptake

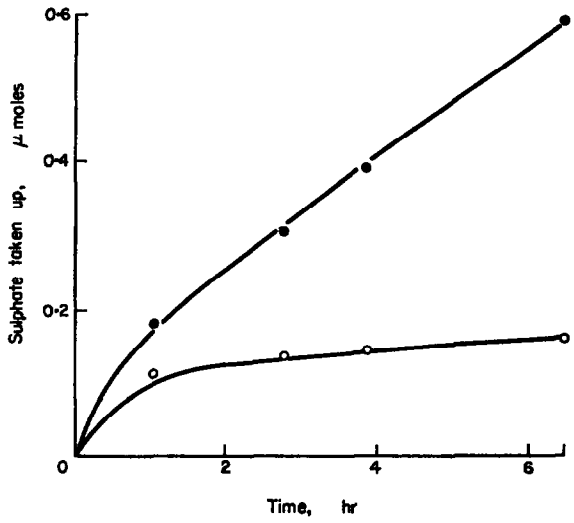

FIG. 1. INHIBITION OF SULPHATE UPTAKE.

Samples of 30 three-day-washed beet slices were shaken in 3 ml 1 mM $Na_2{}^{35}SO_4$ with (○) or without (●) 5 mM D-serine. The uptake of sulphate was measured as described in the text. The figures are averages derived from duplicate flasks.

TABLE 2. EFFECT OF D- AND L-AMINO ACIDS ON SULPHATE AND OXYGEN UPTAKE.

Samples of 30 four-day-washed beet slices were pretreated in 5 ml water or amino acid solution for 3 hr at 25°. The slices were then rinsed in water for 1 min, blotted and shaken in 5 ml 1 mM $Na_2{}^{35}SO_4$ for a further 3 hr. Other samples of slices from the same batch were similarly pretreated and rinsed but then placed in 3 ml 1 mM Na_2SO_4 in Warburg flasks and the oxygen uptake measured for 3 hr at 25°. Results are averages derived from duplicate flasks

Pretreatment solution	Concentration (mM)	Amino acid taken up (μ moles/30 slices)	$^{35}SO_4$ taken up after pretreatment (μm moles/30 slices)	O_2 taken up after pretreatment (μl/hr/g fresh wt.)
Water	—	—	342	79
D-Serine	0·5	1·4	305	—
	1·0	2·3	201	84
	5·0	5·6	152	79
	50·0	—	132	—
D-Alanine	1·0	4·2	240	97
	5·0	9·6	168	124
D-Threonine	1·0	2·8	264	84
	5·0	7·8	178	86
L-Serine	5·0	6·2	357	—
L-Alanine	5·0	10·0	352	—
L-Threonine	5·0	11·8	312	—
Chloramphenicol	6·2	—	145	—

of these inhibitors is considered. The L-isomers of serine, alanine and threonine have no significant effect on sulphate uptake, although they themselves are taken up by the slices at rates closely similar to those of the D-isomers (Table 2).

The effect of chloramphenicol on salt uptake has been compared with that of D-serine, and at a similar concentration, chloramphenicol produces a comparable reduction in ion uptake with beet and carrot slices (Tables 1 and 2). With excised pea roots the antibiotic is far more effective than D-serine and almost completely suppresses the uptake of sulphate (Table 4).

3. *Further Investigations of the* D-*Serine Effect*

A number of observations bearing on the mechanism of the inhibition by D-serine have been made.

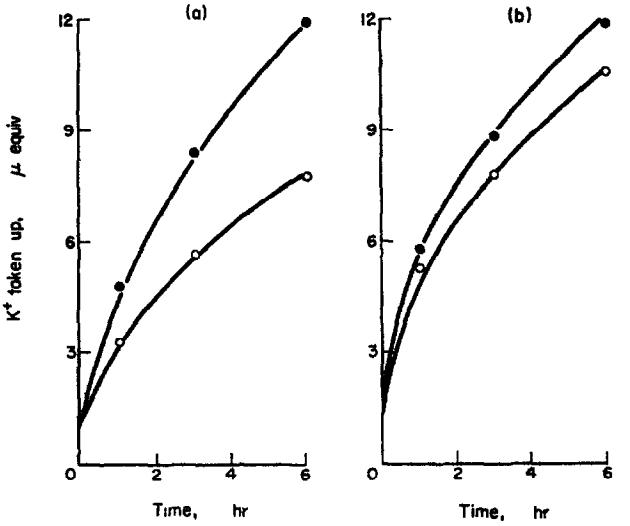

Fig. 2. Removal of D-serine effect by washing.

A. Samples of 30 four-day-washed beet slices were shaken in either 5 mM KCl (●) or 5 mM KCl+1 mM D-serine (○) at 25°.

B. The samples of slices used in A were washed in aerated distilled water for 16 hr and both sets placed in 5 mM KCl.

The figures are averages derived from triplicate flasks.

(*a*) *Efflux.* An inhibitor may alter the permeability of a tissue so that an apparent reduction in the influx of an ion is due to an increased efflux. This explanation has been ruled out for D-serine by an experiment in which beet slices were allowed to accumulate labelled sulphate for 3-hr, rinsed briefly in water, and then placed in water, D-serine, or a solution of unlabelled sulphate. About 5 per cent of the accumulated label rapidly appears in the external solution but there is no difference between the amounts released in the three cases.

(*b*) *Reversibility.* The inhibitory effect of treatment with D-serine is largely removed by washing the treated slices in water for 16 hr (Fig. 2), indicating that no permanent damage to the tissue has occurred.

(*c*) *Competition.* In view of the structural dissimilarity between D-serine and the ions it is unlikely that the inhibition by D-serine is caused by competition between D-serine and the ions for "absorption sites" in the tissue. However, the possibility of competition was tested

by measuring the uptake of D-serine in the presence and absence of various ions. It was found that the presence of potassium chloride, potassium nitrate, potassium sulphate, potassium dihydrogen phosphate and sodium sulphate in 5 mM concentration did not affect the rate of uptake of D-serine (1 mM) by beet slices. In each case 1·4–1·6 μmoles of D-serine were taken up in 1 hr, and 4·1–4·3 μmoles in 6 hr.

Pretreatment of tissue with D-serine is effective in causing inhibition of salt uptake (Table 2). The fact that D-serine does not have to be present in the external solution at the same time

TABLE 3. SALT RESPIRATION OF BEET SLICES IN PRESENCE OF D-SERINE.

Samples of five-day-washed beet slices were shaken in 2·5 ml water or 2 mM KCl in Warburg flasks for 1 hr at 25° and the rate of oxygen uptake measured. Water, D-serine and/or KCl were then tipped from the side-arms to give the final concentrations shown and the oxygen uptake measured for a further 2 hr. The rates of uptake are expressed as a percentage of one of the control flasks

Flask	Medium in flask	O_2 uptake before tipping	Solution tipped	O_2 uptake after tipping
1	Water	100	Water	96
2	Water	95	D-Serine 5 mM	95
3	KCl 2 mM	127	KCl 2 mM	130
4	KCl 2 mM	120	KCl 2 mM + D-Serine 5 mM	118

TABLE 4. UNCOUPLING OF SALT UPTAKE FROM RESPIRATION IN PEA ROOTS.

Samples of 12 pea root apices from four-day-old seedlings were shaken in 5 ml 1 mM $Na_2{}^{35}SO_4$ with and without 5 mM D-serine or 6·2 mM chloramphenicol for 3 hr at 25° and the sulphate uptake estimated as described in the text. Other samples of 12 apices were shaken in 3 ml 1 mM Na_2SO_4 in Warburg flasks with and without the same concentrations of inhibitors and the oxygen uptake measured for 2 hr at 25°. The figures are averages derived from duplicate flasks

Solution	$^{35}SO_4$ taken up (μm moles/12 roots)	O_2 taken up (μl/hr/g fresh wt.)
Na_2SO_4	501	554
Na_2SO_4 + D-serine	361	574
Na_2SO_4 + chloramphenicol	44	572

as the salt to cause inhibition also suggests that there is no competition between inhibitor and ions.

(*d*) *Respiration.* It is possible that D-serine may affect salt uptake through interference with respiration. Table 2 compares the effect of D-serine, D-alanine and D-threonine on both the sulphate uptake and the oxygen uptake of beet slices. It can be seen that D-serine and D-threonine have no significant effect on the uptake of oxygen at concentrations which strongly inhibit the uptake of sulphate. D-Alanine stimulates the oxygen uptake, a finding previously reported for carrot slices.[8] Table 3 shows that D-serine affects neither the respiration of beet slices in water nor the increased respiration that occurs in the presence of potassium chloride.

The uncoupling of salt uptake from respiration by D-serine could also be demonstrated with excised pea roots (Table 4). With this tissue the effect of chloramphenicol is much more

[8] L. M. BIRT and F. J. R. HIRD, *Biochem. J.* 70, 277 (1958).

marked than that of D-serine; the antibiotic inhibits the sulphate uptake by over 90 per cent without significantly affecting the accompanying oxygen uptake.

DISCUSSION

The results presented establish that D-serine is a general inhibitor of cation and anion uptake by higher plant tissues. In many respects the nature of this inhibition by D-serine is similar to that produced by chloramphenicol, the major difference being the rapid disappearance of the chloramphenicol inhibition on rinsing storage tissue slices.[4] This difference is not found with pea roots, where pretreatment with chloramphenicol produces the same marked inhibition of sulphate uptake as does the addition of chloramphenicol to the salt solution (unpublished work).

A most interesting observation is the absence of effect of D-serine on the oxygen uptake of plant tissues both in the presence and absence of salt (Tables 2, 3, 4). Other inhibitors of salt uptake, apart from chloramphenicol, either stimulate (e.g. 2,4-dinitrophenol) or inhibit (e.g. cyanide) the uptake of oxygen.[1,9] This distinction suggests that D-serine, like chloramphenicol, is acting at some point between the supply of energy from respiration and the utilization of this energy in the accumulation of salt and not, as do other inhibitors, on the production of this energy. Chloramphenicol is known to be a specific inhibitor of protein synthesis in bacteria,[10] and some evidence has been presented that chloramphenicol inhibits the incorporation of amino acids into the soluble protein of carrot slices.[11] Studies on the effect of both D-serine and chloramphenicol on amino acid uptake and incorporation in beet slices are described in the following paper.[12]

Washed beet slices contain no detectable D-serine (unpublished); D-serine has been isolated from earthworms[13] and leaf-eating insects[14] although the leaves fed to these insects contain none of it.

EXPERIMENTAL

Chemicals

D-Amino acids were obtained from L. Light & Co. Ltd. and L-amino acids from British Drug Houses Ltd.; D-threo-chloramphenicol was obtained from Parke, Davis & Co. Carrier-free sodium [^{35}S] sulphate was obtained from the Radiochemical Centre, Amersham. All the salts used were of "Analar" grade.

Purity of D-*Serine*

The sample of D-serine gave the expected intensity of ninhydrin colour in the Yemm and Cocking procedure[15] and revealed a single ninhydrin-positive spot of the correct R_f in the following four solvents: butan-1-ol–acetic acid–water (4:1:5, v/v, top phase); butan-1-ol–pyridine–water (1:1:1, v/v, top phase); water-saturated phenol; butan-1-ol–methyl ethyl ketone–ammonia–water (5:3:1:1, v/v). D-Serine gave 95–103 per cent of the theoretical oxygen uptake when assayed manometrically with a crude D-amino acid oxidase preparation

[9] H. Beevers, *Respiratory Metabolism in Plants*, Row-Peterson Biological Monographs, 1961.
[10] T. D. Brock, *Bacteriol. Rev.* **25**, 32 (1961).
[11] B. Jacoby and J. F. Sutcliffe, *J. exp. Bot.* **13**, 335 (1962).
[12] R. J. Ellis, *Phytochemistry*, **3**, 221 (1964).
[13] H. Rosenburg and A. H. Ennor, *Biochem. J.* **79**, 424 (1961).
[14] N. G. Srinivasan, J. J. Corrigan and A. Meister, *J. biol. Chem.* **237**, PC3844 (1962).
[15] E. W. Yemm and E. C. Cocking, *Analyst* **80**, 209 (1955).

(Seravac Laboratories Ltd.). The content of L-serine in the D-serine sample, as determined by a manometric assay with *Proteus vulgaris*,[16] was less than 1%.

A sample of D-serine was passed through a column of cation-exchange resin (Zeokarb 225) in the H^+ form. The column was washed with 0·1 N HCl and the D-serine eluted with 0·8 N HCl in 55% ethanol. This procedure separates amino acids from inorganic salts and metals.[17] The eluted D-serine was recrystallized from ethanol and found to inhibit sulphate uptake to the same degree as untreated D-serine.

Plant Tissues

Slices (5 mm dia. × 1 mm thick) of red beetroot, carrot and turnip roots were prepared and washed at 25° as previously described.[6] Peas (*Pisum sativum* var. Alaska) were germinated in running tap water for 2–3 days until the radicles were 1–2 cm long. The seedlings were placed on plastic trays with the radicles immersed in distilled water and left in the dark for a further 2 days. Root tips (15 mm) were excised and rinsed in distilled water just before use.

Measurement of the Uptake of Ions, Amino Acids, and Oxygen

The uptake of sulphate was measured by using $^{35}SO_4$ as previously described,[6] except that N-ethyl maleimide was omitted from the extracting solution. Sodium and potassium uptake was measured by analysing aliquots of the external solution by flame photometry; the uptake of phosphate was measured by a phosphomolybdate method.[18]

Oxygen uptake was measured in Warburg flasks at 25° with 2 N KOH (0·2 ml) in the centre wells and air as the gas phase. At the end of the experiment the slices were rinsed in water, blotted and weighed to obtain the fresh weight.

The uptake of amino acids was measured by applying the ninhydrin procedure of Yemm and Cocking[15] to aliquots (200 μl) of the external solution.

Acknowledgements—Thanks are due to Professor H. A. Krebs, F.R.S., and Professor H. K. Porter, F.R.S., for the provision of laboratory facilities and to the Agricultural Research Council for financial assistance.

[16] P. K. Stumpf and D. E. Green, *J. biol. Chem.* **153**, 387 (1944).
[17] G. C. Mueller, G. Bowman and A. Herranen, *Anal. Chem.* **27**, 1357 (1955).
[18] R. L. Dryer, A. R. Tammes and J. I. Routh, *J. biol. Chem.* **225**, 177 (1957).