UPTAKE OF SMALL PEPTIDES BY THE SCUTELLUM OF GERMINATING BARLEY

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Received 15 April 1977

1. Introduction

During the germination of the barley grain, the proteins in the starchy endosperm, the storage tissue, are hydrolysed into a mixture of small peptides and free amino acids by several endopeptidases and carboxypeptidases [1]. Complete hydrolysis of the peptides is unlikely to occur in the starchy endosperm, because the carboxypeptidases do not act on di- and tripeptides [2] and no other peptidases are present [1]. On the other hand, the scutellum, an absorptive organ responsible for transferring digestion products from the endosperm to the seedling, contains high activities of peptidases which act on small peptides. This led Mikola and Kolehmainen [1] to suggest that a proportion of the protein digestion products might be absorbed as peptides and hydrolysed into free amino acids in the scutellum.

Earlier work in this laboratory and elsewhere has shown that the small intestine of animals and several other animal tissues, the yeast *Saccharomyces cerevisiae*, the mould *Neurospora crassa* and various bacteria including *Escherichia coli* have a specific, active uptake mechanism for peptides that is independent of the uptake mechanisms for amino acids (see [3,4]). No work on peptide transport in higher plants has yet been reported.

The aim of this study was to find out if peptides can be taken up by the scutellum of barley, and if so, to study some characteristics of the uptake mechanism. The work is based on the use of the peptides glycylsarcosine (Gly–Sar), glycylsarcosylsarcosine (Gly–Sar–Sar) and glycylsarcosylsarcosylsarcosine (Gly–Sar–Sar–Sar), wich are very resistant to hydrolysis owing to *N*-methylation of their peptide bonds (see [3,4]). The results show that Gly–Sar and Gly–Sar–Sar are taken up by the scutellum against a concentration gradient by a saturable mechanism that is dependent on metabolic energy. The dipeptide and the tripeptide are apparently taken up by the same mechanism; tetrapeptides may also have some affinity for this mechanism. Some results of the preliminary experiments were briefly described at a recent Ciba Symposium [5].

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2. Materials and methods

Grains of a huskless variety of barley (*Hordeum vulgare* L. cv. Himalaya) were surface-sterilized with sodium hypochlorite (10 g/litre) for 20 min and rinsed several times in sterile distilled water. They were allowed to germinate on agar (7.5 g/litre) in sterile petri dishes in the dark at 20°C. After 3 days the scutella were separated from the endosperm and plantlet with a scalpel and washed in distilled water stirred by a stream of oxygen for 3 h at room temperature.

In uptake experiments the standard procedure was as follows: Samples consisting of three scutella were weighed (total weight about 18 mg; unaltered after incubation) and incubated for 1 h at 30°C under oxygen in a shaking water bath (140 strokes/min) in

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stoppered 25 ml conical flasks containing 3 ml of 2 mM labelled or unlabelled peptide in 10 mM sodium phosphate buffer, pH 5.2. The scutella were rinsed with distilled water, extracted for 5 min at 100°C with sulphosalicylic acid (60 g/litre) and the extracts were centrifuged. Estimations of [^{14}C]Gly–Sar and [^{14}C]Gly–Sar–Sar in extracts were obtained by mixing 0.5 ml extract with 15 ml dioxane based scintillation solution (120 g naphthaline, 4 g PPO, 50 mg POPOP made to 1 litre with dioxane) and measuring the radioactivity in a liquid scintillation spectrometer (Packard Tricarb model 3380). The amounts of Gly–Sar–Sar–Sar in extracts were estimated by ion-exchange chromatography with an amino acid analyser (Locarte). The amino acid analyser was also used to estimate the amounts of peptides and free amino acids in some tissue extracts and incubation media.

The water content of the scutella was obtained by drying six batches of 10 scutella for 20 h at 105°C, after 'blank' incubations in water for 4 h.

Rates of uptake are expressed as μmol peptide taken up/g initial fresh weight in 1 h. All values for uptake are the means of 4 determinations. Mean values are given ± SEM.

The barley was obtained from the Agronomy Club, Washington State University, Pullman, Washington, USA. [^{14}C]Gly–Sar, [^{14}C]Gly–Sar–Sar and Gly–Sar–Sar–Sar were synthesized by Dr S. Wilkinson (Wellcome Research Laboratories, Beckenham, Kent) (see [6,7]). The other peptides were purchased from Sigma Chemical Company or from BDH Chemicals Ltd.

3. Results

The scutella of barley grains germinated for three days took up [^{14}C]Gly–Sar from 2 mM solution at an initial rate of 10.3 μmol. $g^{-1}.h^{-1}$ (fig.1). The rate of uptake remained unchanged for at least 1 h, but between 1 h and 9 h it gradually slowed down. Ion-exchange chromatography showed that there was no free glycine or sarcosine in the incubation media and that 91% of the Gly–Sar taken up was present in the tissue in intact form after 1 h incubation and 86% after 9 h incubation. After 1 h incubation in 2 mM Gly–Sar, the concentration of the intact peptide in

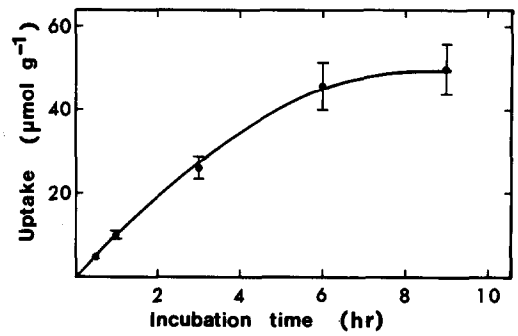

Fig.1. Time course of uptake of [^{14}C]Gly–Sar by the scutella of germinating barley.

the tissue water was 11.9 mM and after 9 h it was 54.5 mM. The corresponding concentration ratios (tissue water : medium) were 6 : 1 at 1 h and 32 : 1 at 9 h. The water content of the scutella was 78.8 ± 0.7% of the fresh weight, and it was assumed that the peptide was evenly distributed in the tissue water.

The 1 h uptake of [^{14}C]Gly–Sar under N_2 was 7.5 ± 1% of the control value, and the tissue water : medium concentration ratio after 1 h was 0.5 : 1.

To see if the uptake was dependent on sodium ions, the uptake was determined from [^{14}C]Gly–Sar solutions made up in water (pH 5.5) and in 10 mM sodium phosphate (pH 5.2). The uptake was the same from both solutions.

The effect of concentration of [^{14}C]Gly–Sar in the medium on the rate of uptake is shown in fig.2. To estimate the amount of Gly–Sar taken up by non-mediate uptake, the data relating uptake of [^{14}C] Gly–Sar to concentration were treated as if unlabelled substrate were acting as a competitive inhibitor of uptake of the labelled form [8], taking the concentration of the labelled form as 2 mM. The

$$\frac{v_o}{v_o - v_i} / \frac{1}{I}$$

plot [7,9], where v_o = uptake in the absence of inhibitor, v_i = uptake in the presence of inhibitor and I = concentration of inhibitor, was used to extrapolate the inhibitory effect to an infinitely high concentration of inhibitor. At such a concentration, mediated uptake would be expected to be completely suppressed, leaving only non-mediated uptake. Knowing the

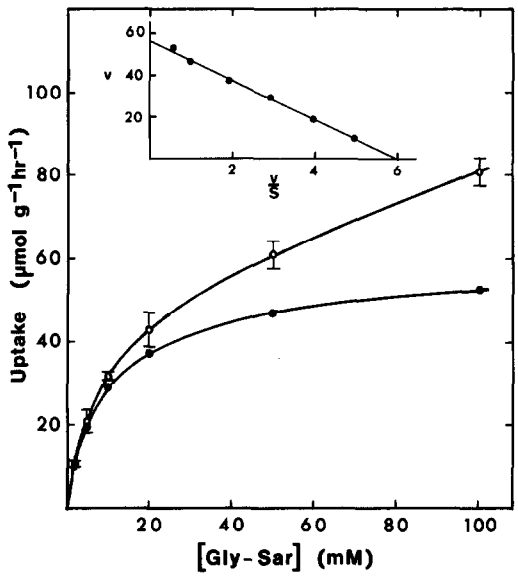

Fig.2. Effect of substrate concentration on uptake of [^{14}C] Gly–Sar by the scutella of germinating barley. (–○–) Original values ± SEM; (–●–) values corrected for non-mediated uptake. The insert represents the $v/\frac{v}{S}$ plot of the corrected values.

magnitude of the non-mediated uptake at a concentration of 2 mM (0.53 μmol.g^{-1}.h^{-1}), the non-mediated uptake could be calculated for any other concentration, since it would be expected to be directly proportional to concentration. After the original values had been corrected for non-mediated uptake a rectangular hyperbola was obtained

and $\frac{1}{v}/\frac{1}{S}$ and $v/\frac{v}{S}$

Table 1
Effect of glycine and some glycyl peptides (40 mM) on the uptake of [^{14}C]Gly–Sar (2 mM) by scutella of germinating barley

	Uptake	
	μmol.g^{-1}.h^{-1}	% Control value
Control	8.83 ± 0.76	
+ Gly	9.69 ± 0.46	110
+ Gly–Gly	0.77 ± 0.05	8.7
+ Gly–Gly–Gly	0.71 ± 0.08	8.0
+ Gly–Gly–Gly–Gly	4.91 ± 0.33	55

plots gave a straight line [8]. This indicates that uptake conformed to Michaelis-Menten kinetics. K_t for uptake of Gly–Sar was 9.6 mM and V_{max} was 57 μmol.g^{-1}.h^{-1} (calculated from the $v/\frac{v}{S}$ plot).

To study the specificity of the uptake mechanism the uptake of [^{14}C]Gly–Sar from 2 mM solution was measured in the presence of 40 mM glycine and also 40 mM di-, tri- and tetraglycine. Free glycine did not inhibit the uptake of Gly–Sar, di- and triglycine inhibited it almost completely, the tetraglycine gave a 45% inhibition (table 1).

The ability of the scutella to take up tripeptides was investigated using [^{14}C]Gly–Sar–Sar. The rate of uptake from 2 mM solution was 3.6 μmol.g^{-1}.h^{-1} and it remained unchanged for at least 3 h (table 2). After 3 h incubation the concentration of the tripeptide in the tissue water was 11.1 mM and the concentration gradient was 5.5 : 1. Uptake of Gly–Sar–Sar was almost totally inhibited by 40 mM diglycine. Ion-exchange analysis indicated that no hydrolysis of the tripeptide had occurred in the scutella.

Table 2
1 h and 3 h uptake of [^{14}C]Gly–Sar–Sar (2 mM) and unlabelled Gly–Sar–Sar–Sar (2 mM) by the scutella of germinating barley, and the effect of diglycine (40 mM) on 3 h uptake

	Incubation time (h)	Uptake (μmol.g^{-1}.h^{-1}) No addition	+ Gly–Gly
Gly–Sar–Sar	1	2.81 ± 0.06	–
	3	8.80 ± 0.39	1.41 ± 0.08 (16%)
Gly–Sar–Sar–Sar	1	0.27 ± 0.02	–
	3	0.95 ± 0.05	0.81 ± 0.04 (85%)

The inhibited values as % control values are shown in brackets

The tetrapeptide Gly–Sar–Sar–Sar was taken up very slowly, the rate of uptake from 2 mM solution being only 0.3 μmol.g^{-1}.h^{-1} and even after 3 h the concentration in the tissue water was lower than in the medium (table 2). The uptake of the tetrapeptide was not significantly inhibited by 40 mM diglycine ($P > 0.05$).

4. Discussion

The results suggest that the dipeptide Gly–Sar is taken up by barley scutellum by an active peptide transport mechanism which is independent of the transport mechanism(s) for free glycine. Since a dipeptide almost completely inhibited the uptake of a tripeptide and vice versa, it seems that the same mechanism transports tripeptides too. The tetrapeptide Gly–Sar–Sar–Sar is transported by this mechanism very slowly if at all, but the mechanism may have some affinity for tetraglycine. Accumulation of this tetrapeptide against a concentration gradient was not demonstrated.

The chain-length specificity of the peptide transport mechanism in barley seems to be similar to that in the mammalian intestine [6]. Both transport di- and tripeptides but tetrapeptides are either not transported or transported only to a small extent. In contrast to the peptide transport mechanism in animal tissues (see [3]), the mechanism in barley is independent of sodium ions. This seems to be a general feature of plant transport mechanisms for sugars and amino acids [10].

E. coli has at least two separate transport systems for peptides: one is specific for dipeptides, the other transports peptides containing from 2 to at least 6 amino acid residues [11]. The chain-length specificity of the peptide transport mechanism in barley does not resemble the specificity of the two mechanism in *E. coli*.

Together with previous work on other groups of organisms, the present results, providing evidence for transport of peptides in higher plants, show that peptide transport is demonstrable in representatives of all the main forms of life.

Acknowledgements

The visit of T.S. in London was supported by grants from the Finnish Cultural Foundation and Emil Aaltonen Foundation. T.S. is a Research Assistant of the Finnish National Research Council for Sciences.

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