INHIBITION OF TRANSPORT OF GLYCYLSARCOSINE BY SOME OTHER DIPEPTIDES IN THE SCUTELLUM OF GERMINATING BARLEY GRAIN

Tuomas SOPANEN

Biotechnical Laboratory, Technical Research Centre of Finland, PO Box 192, 00121 Helsinki 12, Finland

Received 15 October 1979

1. Introduction

The scutella of germinating barley grains take up small peptides rapidly from incubation media by an active, carrier-mediated transport process [1,2]. The uptake of one dipeptide, glycylglycine, was strongly inhibited by all of the 11 other dipeptides tested, and the inhibition by one of these, glycylsarcosine (Gly–Sar), was competitive and complete when extrapolated to an infinitely high concentration of the inhibitor. We therefore suggested that all of these dipeptides might be taken up by a common transport system(s). The uptake of several di- and tripeptides by imbibed barley embryos has also been shown to be inhibited by other small peptides [3,4] and (supposing that the peptides acted as competitors) it was suggested that there was only a single peptide transport system in barley embryos.

This work shows that the uptake of Gly–Sar is inhibited by all of the 5 other dipeptides tested (Gly–Gly, Pro–Pro, Ala–Ala, Leu–Leu, Arg–Arg). Extrapolation of their effect to an infinitely high concentration indicated that all of these 5 dipeptides would completely inhibit the uptake of Gly–Sar (4 mM). However, only Gly–Gly acted as a pure competitive inhibitor while the inhibitions caused by the other peptides were, unexpectedly, of the mixed type (Pro–Pro) or almost purely non-competitive (Ala–Ala, Leu–Leu, Arg–Arg). The results show that the inhibition effects in peptide uptake in barley scutella are more complicated than anticipated. Therefore, mere inhibition of the uptake of one peptide by another should not be taken as a conclusive proof for a common carrier.

2. Materials and methods

The experimental procedure was similar to that in [2]. Grains of a huskless variety of barley (*Hordeum vulgare* L. cv. Himalaya) were surface-sterilized and allowed to germinate in aseptic conditions on agar gel in the dark at 20°C for 3 days. The scutella were removed, prewashed for 3 h, and samples of 4 scutella were weighed and incubated at 30°C for 1 h in 10 mM sodium citrate buffer (pH 4.5) containing [^{14}C]-Gly–Sar alone or with another peptide. The scutella were then rinsed with water, immersed directly into the scintillation cocktail [5], and shaken vigorously. The radioactivities were measured after 1–5 days' storage at 5°C. In earlier experiments, peptides were extracted from the scutella with hot sulphosalicylic acid solution for measurement of radioactivity, but this was later found to be unnecessary.

The linearity of the uptake during the 1 h incubation was tested for 2 mM Gly–Sar. In the other experiments the uptake time of 1 h was used routinely. Rates of uptake are expressed as μmol peptide taken up/g fresh wt in 1 h. All values are means of 4 determinations. The standard error of the mean was usually within 10% of the mean. All experiments were done at least twice with essentially similar results.

The barley was obtained from the Agronomy Club, Washington State University, Pullman, Washington. [^{14}C]Gly–Sar was synthesized by Dr S. Wilkinson [6]. The other peptides were purchased from Bachem Feinchemikalien AG; they were all of L–L configuration; Arg–Arg was supplied as acetate.

3. Results

All of the 5 dipeptides tested (Gly–Gly, Pro–Pro, Ala–Ala, Leu–Leu, Arg–Arg) inhibited the uptake of Gly–Sar by barley scutella. When the uptake of 4 mM Gly–Sar was estimated in the presence of increasing concentrations of these peptides, the inhibitions at the highest concentrations tested were 80–95%. Extrapolation of the inhibitory effect to an infinitely high concentration of the inhibitor by plotting $(v_o/(v_o - v_i))/(1/I)$ [6–8] indicated that the uptake of Gly–Sar (4 mM) would be completely inhibited at an infinitely high concentration of each of the 5 dipeptides (fig.1).

The inhibitions were next measured using a single concentration of the inhibitory dipeptide and increasing concentrations of Gly–Sar, and the results were plotted as $v/(v/S)$ [9,10]. The plots showed that Gly–Gly inhibited the uptake of Gly–Sar in a strictly competitive manner (fig.2A), with K_i 3 mM. The K_m value for Gly–Sar in this experiment was 8.1 mM, which compares reasonably well with the value of 9.6 mM obtained in [1].

When the 4 other dipeptides were tested, the inhibitions were of the mixed type (simultaneous increase in app. K_m and decrease in V_{max}). With Pro–Pro (fig.2A) the competitive component was

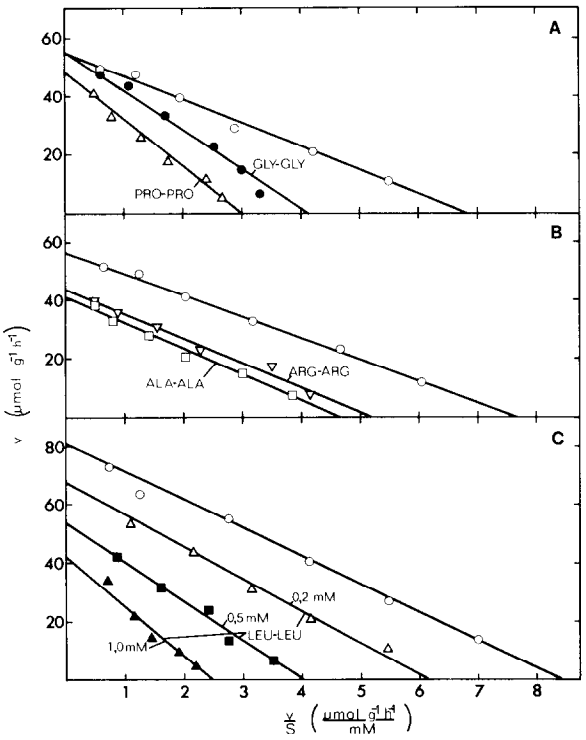

Fig.2. Effect of 5 dipeptides at constant concentration on the uptake of Gly–Sar by barley scutella. The uptake of Gly–Sar (2–80 mM) was estimated alone or in the presence of the following dipeptides: (A) 2 mM Gly–Gly, 1 mM Pro–Pro; (B) 0.5 mM Ala–Ala, 0.2 mM Arg–Arg; (C) 0.2, 0.5 and 1 mM Leu–Leu. The $v/(vS)$ plots of the experimental values are shown.

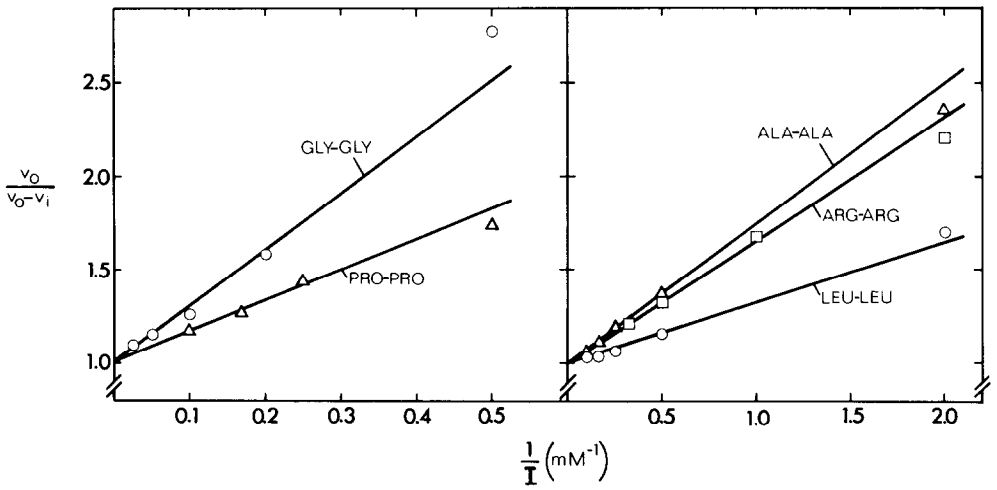

Fig.1. Effect of 5 dipeptides at increasing concentrations on the uptake of Gly–Sar by barley scutella. The uptake of 4 mM Gly–Sar was estimated in the presence of Gly–Gly (2–40 mM), Pro–Pro (1–6 mM), Ala–Ala (0.5–10 mM), Arg–Arg (0.2–3 mM) and Leu–Leu (0.5–10 mM). The $(v_o/(v_o - v_i))/(1/I)$ plots of the values are shown (v_o, rate of uptake in the absence of inhibitor; v_i, the same in the presence of inhibitor; I, inhibitor concentration).

prominent, but with Ala–Ala, Arg–Arg (fig.2B) and Leu–Leu (fig.2C) the inhibitions were mainly non-competitive with only a minor competitive component (large decrease in V_{max} compared to small increase in app. K_m).

4. Discussion

In previous work on amino acid transport in higher plants, it has been a general practice to interpret the inhibition of the uptake of one amino acid by another as evidence for a common transport system. Some justification for this assumption has been given by the fact that, where this type of inhibition has been studied, it has been found to be competitive (e.g. [11–14]). A similar assumption has also been made in the studies on the uptake of peptides by barley scutella or whole embryos [1–4], and on this basis a common, single mechanism for uptake of oligopeptides has been proposed [3]. However, the present data reveal that the situation is more complicated.

On the basis of the present and previous results [2] a common carrier(s) for Gly–Sar and Gly–Gly is strongly implicated. Both dipeptides act as pure competitive inhibitors of uptake of the other and extrapolations indicate complete inhibitions at an infinitely high concentration of the inhibitor. Moreover, the K_i values for both peptides are within experimental error the same as the corresponding K_m values for the uptake.

The mixed inhibitions caused by the other dipeptides are more difficult to explain. In enzyme kinetics the most common interpretation of mixed inhibitions is that the presence of inhibitor or substrate affects the binding affinity of the other species although they bind at separate sites [15]. However, especially in transport across membranes, mixed inhibition by a substrate analogue could also be the result of two parallel effects:

(i) Competitive inhibition would result if the substrate analogue were to compete with the substrate of the active site facing the outside of the membrane (without regard to whether the analogue were transported or not).

(ii) Non-competitive (or mixed) inhibition might result if the inhibitor binded, e.g., to another site on the carrier or to the substrate binding site when this was facing the inner side of the membrane (transinhibition, see [16]).

If the inhibitor exerted its effect from within the cell, a separate carrier for the inhibitor would be implicated, because an infinitely high substrate concentration does not prevent the inhibition although it should totally inhibit transport of the inhibitor by the same carrier.

Thus, the present data do not rule out the possibility of a common carrier for all oligopeptides in the barley scutellum. However, they suggest rather complicated mechanisms and interactions and therefore a common carrier cannot be assumed on the basis of mere inhibition.

Acknowledgements

My sincere thanks are due to Professor D. M. Matthews for the labelled Gly–Sar and for reading the manuscript, to Professor J. Milola for stimulating discussions at all stages of the work, to Mr M. J. Bailey, BSc for checking the language, and to Ms T. Ahokas and Ms L. Harju for skillful technical assistance. The author is a Research Assistant of the National Council for Sciences, and the work was supported by grants from the same council.

References

[1] Sopanen, T., Burston, D. and Matthews, D. M. (1977) FEBS Lett. 79, 4–7.
[2] Sopanen, T., Burston, D., Taylor, E. and Matthews, D. M. (1978) Plant Physiol. 61, 630–633.
[3] Higgins, C. F. and Payne, J. W. (1978) Planta 138, 217–221.
[4] Higgins, C. F. and Payne, J. W. (1978) Planta 142, 299–305.
[5] Wiegman, T., Woldring, M. G. and Pratt, J. J. (1975) Clin. Chim. Acta 59, 347–356.
[6] Sleisenger, M. H., Burston, D., Dalrymple, J. A., Wilkinson, S. and Matthews, D. M. (1976) Gastroenterology 71, 76–81.
[7] Reinhold, L., Shtarkshall, R. A. and Ganot, D. (1970) J. Exp. Bot. 21, 926–932.
[8] Preston, R. L., Schaeffer, J. F. and Curran, P. F. (1974) J. Gen. Physiol. 64, 443–467.
[9] Neame, K. D. and Richards, T. G. (1972) in: Elementary kinetics of membrane carrier transport, pp. 56–79, Blackwell, Oxford.

[10] Dixon, M. and Webb, E. C. (1964) in: Enzymes, 2nd edn. pp. 315–331, Longman's Green, London.
[11] Cheruel, J., Jullien, M. and Surdin-Kerjan, Y. (1979) Plant Physiol. 63, 621–626.
[12] Lien, R. and Rognes, S. E. (1977) Physiol. Plant. 41, 175–183.
[13] Cheung, Y. -N. S. and Nobel, P. S. (1973) Plant Physiol. 52, 633–637.
[14] Berlin, J. and Mutert, U. (1978) Z. Naturforsch. 33c, 641–645.
[15] Roberts, D. V. (1977) in: Enzyme kinetics, pp. 65–69, Cambridge University Press, Cambridge.
[16] Devés, R. and Krupka, R. M. (1978) Biochim. Biophys. Acta 510, 186–200.