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LACK OF HOMOLOGY BETWEEN THE ALLIIN LYASES OF GARLIC AND ONION*

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Key Word Index—*Allium sativum*; *Allium cepa*; Amaryllidaceae; garlic; onion; homology; enzyme; alliin lyase; immunology.

Abstract—The genus *Allium* contains a number of important food plants whose characteristic flavours and odours are produced by the action of the enzyme alliin lyase (EC 4.4.1.4) on endogenous non-protein *S*-containing amino acids. Previous studies had shown great differences in the purified enzyme from onion (*A. cepa*) and garlic (*A. sativum*). The present study examined the degree of homology of the enzyme protein from these two sources based on its immunological cross-reaction with polyclonal antibodies produced by homogeneous enzyme from each species. The results show no cross-reaction of the garlic enzyme antibodies with the onion enzyme, and an inconsistent amount of cross-reaction of the garlic enzyme with onion antibodies. In addition *N*-terminal amino acid analysis shows a large difference between the purified enzyme from these two sources.

INTRODUCTION

The characteristic flavour and odour associated with a number of common vegetables is a result of the enzymatic degradation of *S*-alkyl-L-cysteines or their sulphoxides present as non-protein secondary amino acids [1]. Upon rupture of the tissue by crushing or slicing, a C–S lyase separated spatially from these amino acids in the intact cell [2] cleaves these compounds to yield the distinctive end-products.

Garlic (*Allium sativum*) and onion (*A. cepa*) are two species in the same genus of the Amaryllidaceae which are obvious examples of the above phenomenon. The precursors of their distinctive odours on crushing are *S*-alkyl-L-cysteine sulphoxides present in both garlic and onion. The enzyme responsible for the breakdown (alliin lyase, EC 4.4.1.4) from each of these species has been purified to homogeneity [3, 4]. The physical and kinetic properties of these two enzymes are quite different, and the question arises as to the degree of homology which exists between enzymes from two species which are so closely related taxonomically.

The present report describes the preparation of polyclonal antibodies in rabbit to each of these alliin lyases, and demonstrating the interaction of the antibody with the enzyme protein used for its production and its degree of cross-reaction with the lyase from the other species. In addition the interaction of the analogous enzyme from leek (*A. porrum*) with each of the antibody species was also examined.

RESULTS AND DISCUSSION

Homogenous garlic and onion alliin lyases were prepared as described in Experimental. A 1% agar solution made up in 0.01 M phosphate buffer (pH 7.2) containing 0.15 M sodium chloride and 0.02% sodium azide was poured into each of several petri dishes (25 ml each). In a 6 mm hole bored in the centre of the plate, 30 μl of the antisera to either the garlic or onion enzyme was placed. In the wells surrounding the centre well aliquots of different enzyme preparations were placed and immunodiffusion carried out. The results of typical experiments are shown in Fig. 1.

The garlic antiserum (Fig. 1a) appears to react only with garlic enzyme protein, i.e. with the protein used to produce the antibody. The onion results (Fig. 1a, b, c) show some possible cross-reaction with denatured garlic enzyme (Fig. 1c). In the latter case the enzyme had been in storage for several weeks longer and it is conceivable that some degradation had occurred which might have allowed exposure of segments of the enzyme not normally available to the antibody. This is also suggested by the *N*-terminal analysis of the garlic enzyme described below.

Another feature of interest on these plates is the appearance of a second band trailing slightly behind the most prominent one in well A in Fig. 1b,c. A possible explanation of these results might be the breakdown or dissociation of the onion enzyme during the diffusion period giving rise to other molecular species able to interact with the antibody. In the case of the SDS-urea treated onion enzyme, the onion enzyme was in its monomer form in every case, and only one reactive band is found.

The results are consistent with previous work [3, 4] which shows that the onion alliin lyase differs markedly from the garlic enzyme in M_r, pH optimum, and the composition of the carbohydrate moiety. We have also found a large difference in the *N*-terminal amino acid

* Part 5 in series 'The C–S Lyases of Higher Plants'. For part 4 see ref. [4].

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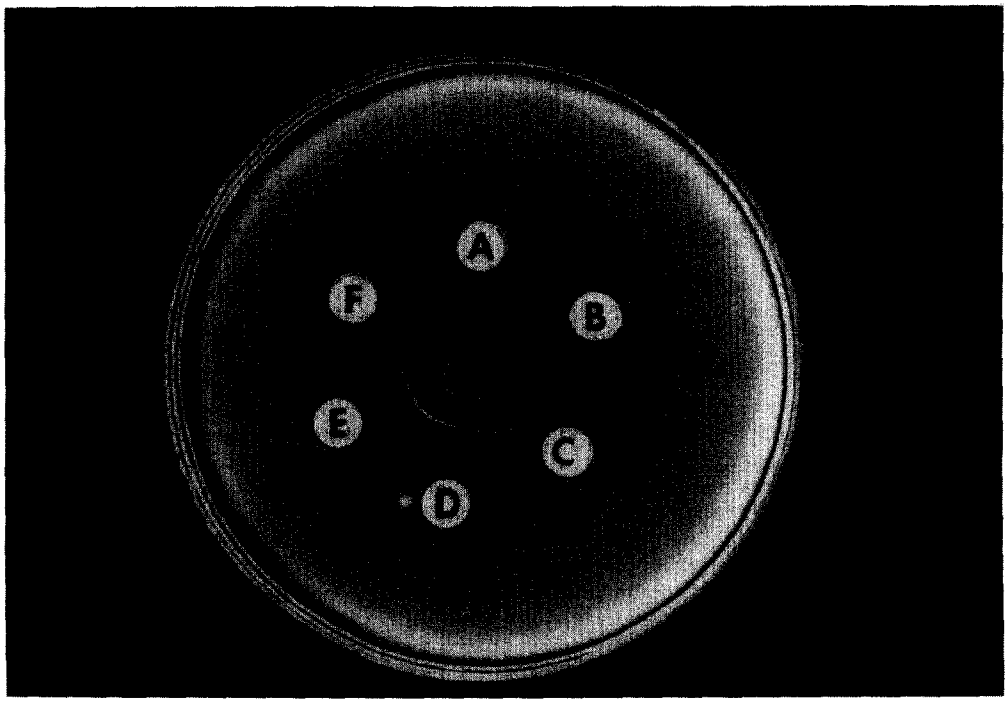

Fig. 1a. Immunodiffusion of garlic and onion alliin lyases against antiserum to homogeneous garlic enzyme. The wells contained the following constituents; A: homogeneous onion lyase; B: onion enzyme treated with 6 M urea; C: onion enzyme treated with 6 M urea + 0.1% Na dodecylsulphate (SDS); D: homogeneous garlic enzyme; E: garlic enzyme treated with 6 M urea; F: alliin lyase from leek (*A. porrum*). The centre well contained antiserum to the garlic enzyme.

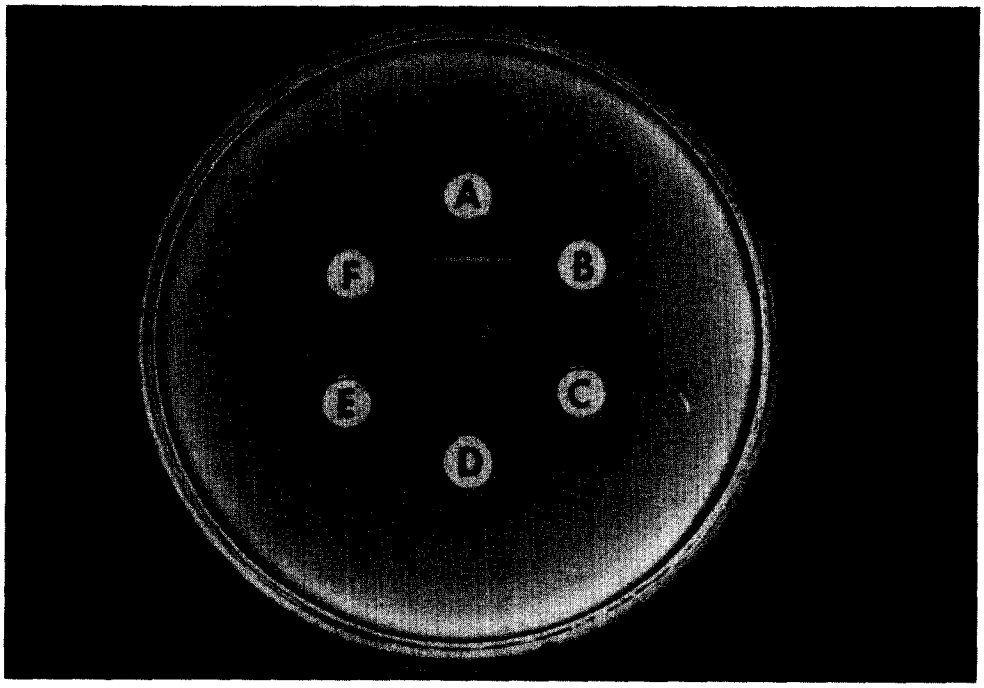

Fig. 1b. Immunodiffusion of garlic and onion alliin lyases against antiserum to homogeneous onion enzyme. Contents of wells A–F are the same as Fig. 1a. Antiserum to the onion enzyme was in the centre well.

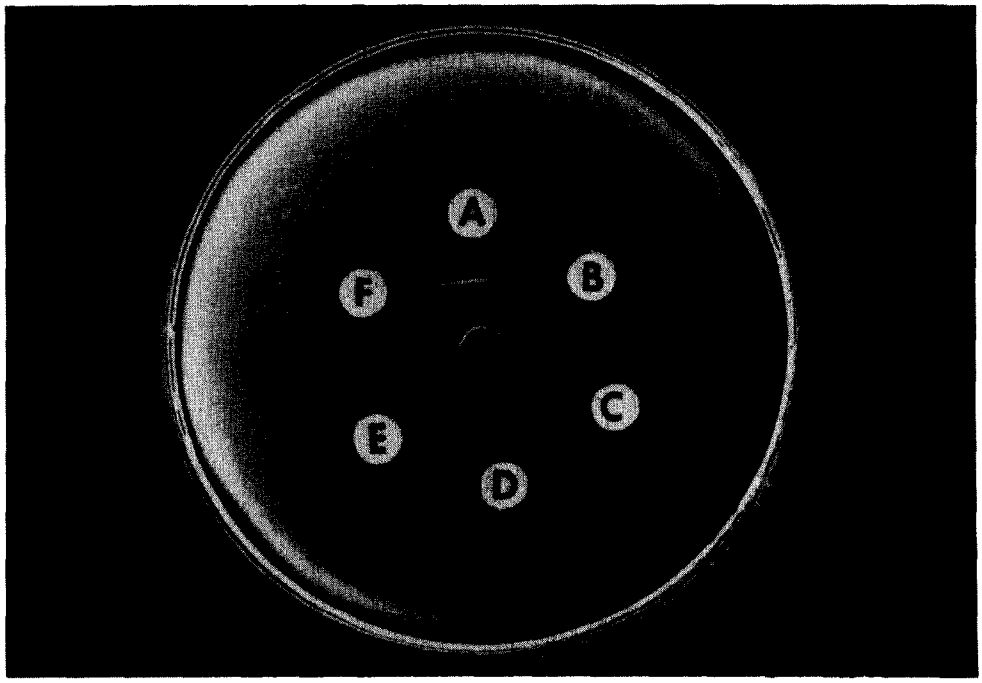

Fig. 1c. Identical with Fig. 1b except enzymes had been stored for two more weeks at −15°.

analysis. The garlic enzyme had the *N*-terminal sequence of lysine, methionine, threonine and tryptophan. On storage, different secondary *N*-terminal sequences in much smaller amounts would appear. These were apparently due to degradation products of the native enzyme since these latter sequences differed with the same preparation with time. The main sequence above was always the same. The *N*-terminal sequence of the onion enzyme could not be determined by the standard method, and is possibly blocked by an *N*-acyl group.

The results obtained indicate that the enzymes from garlic and onion have very little homology in structure. However, even though the holoenzymes differ in M_r, the subunit sizes are quite similar. It must also be borne in mind that these enzymes are glycoproteins, and the composition of the carbohydrate moieties are quite different in each case. Since polyclonal antibodies were produced in the present study, the carbohydrate portion could have contributed the major antigenic determinants, and the protein sequences of the respective monomers might be identical or very similar.

EXPERIMENTAL

Enzyme preparation. Homogeneous garlic and onion alliin lyase were prepared by the procedure of ref. [4]. The leek enzyme was a gift from T. Won, and was essentially homogeneous by disc gel electrophoresis analysis.

Preparation of antibodies. Antisera to each of the lyases was produced in individual rabbits by the technique of ref. [5]. Preimmunization sera from each rabbit was collected prior to the initial injection of the enzyme protein. Injections of various amounts of enzyme protein (maximum 500 μg) subcutaneously over the shoulder lymph nodes were made on a weekly basis until a positive titre of the blood serum to the enzyme protein was observed by immunodiffusion [6].

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