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SUBUNIT STRUCTURE AND IMMUNOLOGICAL PROPERTIES OF WHEAT CARBOXYPEPTIDASE

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Key Word Index—*Triticum aestivum*, Gramineae, wheat, carboxypeptidase, subunit, immunological properties, amino acid composition, amino and carboxyl terminal

Abstract—Wheat carboxypeptidases I, II, III and IV from wheat seeds with isoelectric points of 4 8, 5 6, 6 0 and 6 5, respectively, were found to be homogeneous by the Ouchterlony double immunodiffusion technique using an antiserum of the enzyme III In a previous paper [1], the native enzyme III (MW = 118 k) was separated into two 58 k subunits (MW = 58 k) and further divided into the 35 k and 25 k fragments (MW = 35 k and 25 k, respectively) The native enzyme III and the 58 k subunit produced a single precipitin line against the antiserum The 35 k and 25 k fragments did not cross-react with the antiserum The amino acid compositions of the 35 k and 25 k fragments were similar to each other Amino-terminal amino acids of the 35 k and 25 k fragments were both glutamic acid Carboxy-terminal groups of the 35 k and 25 k fragments were determined to be –(Gly, Ser)–Glu–OH and –Thr–Pro–Glu–OH, respectively The Ouchterlony double immunodiffusion technique revealed the presence of a common antigen in a carboxypeptidase from wheat seeds and one from germinated wheat Comparison of both enzymes is discussed

INTRODUCTION

Wheat carboxypeptidase, isolated and crystallized from wheat is a recently characterized enzyme with a MW of 118 k and an isoelectric point of 6 0, possessing active Ser [1] Carboxypeptidases which are classified as serine carboxypeptidase (EC 3 4 16 1) have been identified and purified in many plants such as french beans [2], germinated barley [3], germinated cotton seeds [4], watermelon [5], orange leaves and exocarp [6, 7], wounded tomato leaves [8] Wheat carboxypeptidase, as well as other serine carboxypeptidases, has the ability to release most amino acid residues, including that of proline, from the carboxyterminus of the peptides and proteins [1, 9] The values of k_{cat} on wheat carboxypeptidase are dramatically influenced by the amino acid residues, while the structure of the substrate does not appreciably affect the K_m value [10]

Four carboxypeptidases, designated the enzymes I, II, III and IV, with isoelectric points of 4 8, 5 5, 6 0 and 6 5, respectively, and with the same MW of 118 k are present in resting wheat seeds The enzyme III with an isoelectric point of 6 0 was shown to be tetrameric [1] The enzyme III resembles germinated wheat carboxypeptidase [11, 12] in substrate specificity, but apparently differs in MW, isoelectric point and optimum pH Wheat carboxypeptidase is considered to be one of key enzymes playing an important role in the degradation of storage proteins at the initial stage of germination

This paper describes the immunological properties of the isoenzymes, the immunological and physiochemical properties of subunits and comparison between the enzyme from resting wheat seeds and that from germinated wheat

RESULTS

Double diffusion of the antiserum of wheat carboxypeptidase III and each of wheat carboxypeptidases I, II, III and IV on an Ouchterlony plate produced a single precipitin line (Fig 1) The line formed between the enzymes I, II, III and IV against the antiserum completely fused, demonstrating that the enzymes I, II, III and IV were homogeneous in terms of immunological properties

Figure 2 shows the result of double immunodiffusion of wheat carboxypeptidase III, the 58 k, the 35 k and 25 k fragments against the antiserum Wheat carboxypeptidase III and the 58 k subunit produced a single precipitin line, which fused with each other, against the antiserum In another 24 hr, a faint spur formation was observed The 35 k and 25 k fragments did not cross-react with the antiserum Figure 3 shows the star-diagram of the relative

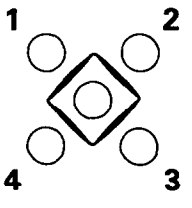

Fig 1 Immunodiffusion cross-reactivity of wheat carboxypeptidases I, II, III and IV against antiserum of wheat carboxypeptidase III Centre well contained antiserum of wheat carboxypeptidase III, and outer wells 1, 2, 3 and 4 contained wheat carboxypeptidase I, II, III and IV, respectively The diffusion was allowed to take place for 24 hr at 25°

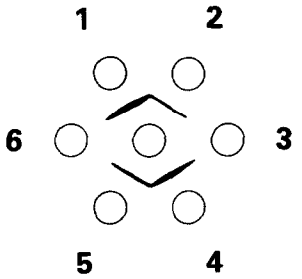

Fig 2 Immunodiffusion cross-reactivity of wheat carboxypeptidase III, the 58 k subunit and the 35 k and 25 k fragments against antiserum of wheat carboxypeptidase III Centre well contained antiserum of wheat carboxypeptidase III, and outer wells contained the following 1 and 4, wheat carboxypeptidase III, 2 and 5, the 58 k subunit, 3, 35 k fragment, 6, 25 k fragment

amino acid composition of the 35 k and 25 k fragments Their amino acid compositions were similar to each other

The *N*-terminal amino acid and the *C*-terminal groups of the 35 k and 25 k fragments are summarized in Table 1 As the *N*-terminal amino acid of both fragments, a large amount of Glu and a trace of Gly were detected by two-dimensional TLC on polyamide sheets The *C*-terminal group of 35 k and 25 k were determined to be –(Gly, Ser)–Glu–OH and –Thr–Pro–Glu–OH, respectively

Figure 4 shows the result of double immunodiffusion of wheat carboxypeptidase III, the 58 k subunit and the crude carboxypeptidase preparation from germinated wheat against the antiserum The crude enzyme preparation from germinated wheat formed a precipitin line against the antiserum The precipitin line between the enzyme III and the crude germinated wheat enzyme preparation partially fused, and a spur was formed towards the well containing the enzyme III The precipitin line between the 58 k submit and the crude germinated wheat enzyme preparation did not fuse at all, though the reaction between the 58 k subunit and the antiserum was faint

DISCUSSION

Wheat carboxypeptidases I, II, III and IV cross-reacted immunologically with the antiserum of the enzyme III (Fig 1) We reported in the previous paper [1] that the four enzymes assumed a mixed crystal form They seem to have the same antigens and to be almost analogous in protein structure

Native wheat carboxypeptidase III separated into two 58 k subunits upon the treatment of SDS, followed by complete inactivation The 58 k subunit was divided into the 35 k and 25 k fragments by further adding 2-mercaptoethanol [1] The Ouchterlony double diffusion test (Fig 2) revealed that the higher order structure of the 58 k subunit was partially maintained and those of the 35 k and 25 k fragments were completely destroyed Hasilik and Tanner [13] reported that an antiserum of carboxypeptidase Y [14], which can be raised in rabbits, reacted with the sugar moieties of the enzyme As wheat carboxy-

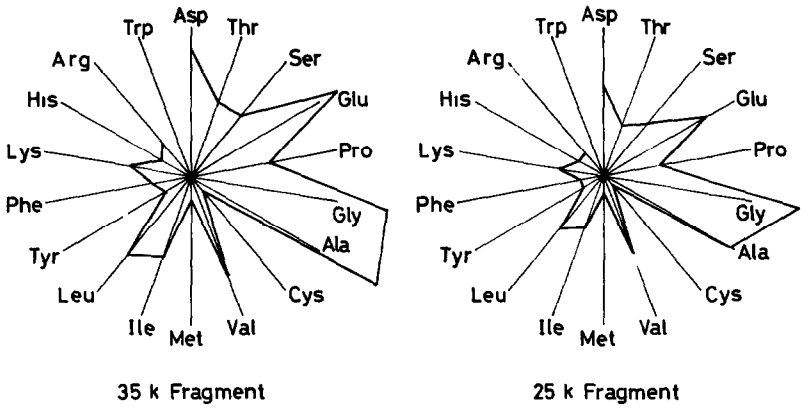

Fig 3 Star-diagram of the relative amino acid composition of the 35 k and 25 k fragments

Table 1 The *N*-terminal amino acids and the *C*-terminal groups of the 35 k and 25 k fragments

	MW	*N*-terminal amino acid	*C*-terminal group
35 k fragment	35 k	Glu ≫ Gly	–(Gly, Ser)–Glu–OH
25 k fragment	25 k	Glu ≫ Gly	–Thr–Pro–Glu–OH

N-Terminal amino acids were determined by the dansyl chloride method *C*-Terminal groups were analysed by the method of carboxypeptidase digestion

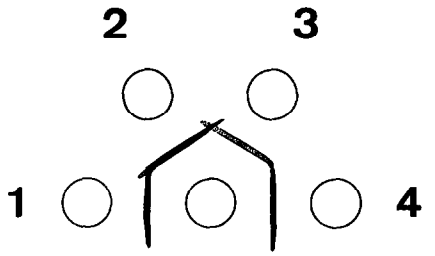

Fig 4 Immunodiffusion cross-reactivity of wheat carboxypeptidase, the 58 k subunit and the crude carboxypeptidase preparation from germinated wheat against antiserum of wheat carboxypeptidase III Centre well contained antiserum of wheat carboxypeptidase III Outer wells contained the following 1 and 4, wheat carboxypeptidase III, 2, the crude carboxypeptidase preparation from germinated wheat, 3, the 58 k subunit The diffusion was allowed to take place for 24 hr at 25°

peptidase is a glycoprotein [1], it is considered that an antiserum of wheat carboxypeptidase III reacted with the 58 k subunit

In *N*-terminal amino acid analyses of the 35 k and 25 k fragments (Table 1), Glu was observed as a major component and Gly, as a minor one We consider that the *N*-terminal amino acid of the 35 k and 25 k fragments was Glu, since the minor component of Gly might have originated from the proteolysis of the enzyme by other proteolytic enzymes

In the Ouchterlony double immunodiffusion test (Fig 4), the native wheat carboxypeptidase from wheat seeds and the germinated wheat enzyme preparation partially fused One component or another except carboxypeptidase in the crude enzyme preparation from germinated wheat seeds seems to react with the antibody corresponding to it in an antiserum of wheat carboxypeptidase III On the other hand, no fused precipitin between the germinated enzyme preparation and the 58 k subunit was observed at all These results indicate that the native enzyme from wheat seeds and germinated wheat enzyme have a common antigen in molecules, whereas germinated wheat carboxypeptidase and the 58 k subunit did not hold the same antigen in common There are two possibilities in the formation of germinated wheat carboxypeptidase the first is *de novo* synthesis during the germination, and the second is that wheat carboxypeptidase in resting seeds is converted into a germinated enzyme in the initial stage of germination with some modification, followed by the reduction of MW to one half of the initial value The latter case is probable on the basis of the presence of a common antigen between carboxypeptidases from resting wheat seeds and germinated ones

EXPERIMENTAL

Preparation of enzymes Wheat bran from bread and common wheat (*Triticum aestivum* L) was purchased from Nissin Seifun Co Ltd, Tokyo Wheat carboxypeptidase III was prepared according to ref [1] and the enzymes I, II and IV were prepared by the method of CM-cellulose rechromatography [1] The enzyme III was found to be homogeneous by disc electrophoresis at pH 4 and analytical ultracentrifugation The enzymes I, II and IV were proven to be homogeneous by CM-cellulose chromatography

Carboxypeptidase from germinated wheat (*Triticum aestivum* L cv Norin 61) was prepared by the method of ref [11] The $(NH_4)_2SO_4$ precipitation of crude enzyme preparation was chromatographed on a Sephadex G-75 column with 0 05 M acetate buffer (pH 4 4) Active fractions of the enzyme were collected and dialysed against the same buffer The dialysed soln was used as a partially purified enzyme preparation This enzyme preparation was confirmed to contain carboxypeptidase of *ca* 60 k in MW originating from germinated wheat but not to contain carboxypeptidase of 118 k in MW originating from resting wheat seeds by the analytical gel filtration method with Sephadex G-100

Double immunodiffusion analyses Rabbit antiserum to pure wheat carboxypeptidase III was prepared according to ref [15] Immunodiffusion experiments were carried out by the Ouchterlony double diffusion technique [16] Ouchterlony plates were prepared on glass sheets using 1 2% agar in buffered saline at 20° for 24 to 48 hr

Amino acid analyses Amino acid analysis was performed by the method of ref [17] Amino acids were analysed with a Hitachi amino acid analyser, Model 834-30 The numbers of amino acid residues of the 35 k and 25 k fragments were calculated for MWs of 35 k and 25 k, respectively

Determination of the N-terminal amino acid and C-terminal group The *N*-terminal amino acid was determined by the dansyl chloride method of ref [18] The detection of dansyl amino acid was performed by 2-D TLC on polyamide sheets Release of the *C*-terminal amino acids from the 35 k and 25 k fragments was performed by digestion with *Penicillum janthinellum* acid carboxypeptidase [19, 20] The released amino acids from the carboxy-terminus were identified by the amino acid analyser

Preparation of the 58 k subunit and the 35 k and 25 k fragments The 58 k subunit and the 35 k and 25 k fragments were prepared by the method of SDS-polyacrylamide gel electrophoresis [21] in 10% gel as mentioned as follows To obtain the 35 k and 25 k fragments, wheat carboxypeptidase III was treated with 1% SDS and 2% 2-mercaptoethanol Then the 35 k and 25 k fragment fractions were cut from the gel and extracted with H_2O The extracted fragments were dialysed against H_2O and freeze-dried The 58 k fragment was prepared in a similar manner to the above, except that the enzyme III was treated only with 1% SDS

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