Note

Effect of Cycloheximide on Protein Breakdown in Germinating Rice Seeds

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Rice grain usually contains 6 to 8% protein on a fresh weight basis. Glutelin is a major reserve protein in rice. It has a large molecular weight (2.0×10^6) and consists of three subunits, I, II and III, linked together by disulfide bonds.¹⁻³ γ Globulin (7S globulin) is also a main reserve protein in rice. It consists of three distinct components, γ_1 , γ_2 and γ_3 globulins. γ_1 Globulin has a very complex subunit composition and has a highly asymmetrical shape, but γ_3 globulin has a simpler subunit composition and a nearly spherical shape. There is little difference between γ_2 and γ_3 globulins.⁴⁻¹⁰

FIG. 1. Growth of Rice during Germination in Presence of Cycloheximide.

Rice seeds were soaked in 200 ml of the cycloheximide solution. 100 seeds of each germinating stage were used for analysis.

○—○, control; △—△, 10 µg/ml cycloheximide and ▲—▲, 20 µg/ml cycloheximide.

There should be some difference in physiological roles between glutelin and γ globulin because they are quite different in physicochemical properties and in localization: glutelin in endosperm protein bodies^{11,12} and γ globulin in the scutellum and aleurone cells.¹⁰ The present paper describes the changes in rice protein contents during germination in the presence of an inhibitor of protein synthesis to clarify their physiological roles and the mechanisms of breakdown.

Rice seeds of a Japonica variety, "Nihonbare," were used in this experiment and placed in a solution containing cycloheximide in the dark at 30°C for 2 to 14 days. Protein was assayed according to the procedure of Lowry *et al.*¹³ The determination of γ globulin was performed by the quantitative immuno-precipitin test described in the previous paper.¹⁰

We reported previously that the contents of γ globulin components decreased at an early stage of germination.¹⁰

FIG. 2. Change in γ_1 Globulin Content during Germination.

Rice seeds were soaked in 200 ml of the cycloheximide solution. 100 seeds of each germinating stage were used for analysis.

○—○, control; △—△, 10 µg/ml cycloheximide and ▲—▲, 20 µg/ml cycloheximide.

We examined whether the breakdown of γ globulin was sensitive to the inhibitor of protein synthesis. Figure 1 shows the growth of rice during germination in the presence of cycloheximide. The growth of roots was strongly suppressed in the presence of cycloheximide. Figures 2 and 3 show the changes in the contents of γ_1 and γ_3 globulins, respectively, during germination in the presence of cycloheximide. Both globulins decreased rapidly during germination, and the breakdown was not affected by 10 to 20 $\mu\text{g}/\text{ml}$ cycloheximide, although a considerable repression was observed of the growth. This result shows that the breakdown of γ globulin is not sensitive to the inhibitor of protein synthesis and suggests that γ globulin components are probably degraded by protease *in situ* or rapid activation of zymogen.

The change in glutelin content in rice grain during germination was followed as shown in Fig. 4. Glutelin was decomposed from the 4th to 12th day of germination. However, only about 60% of the total glutelin was

FIG. 3. Change in γ_3 Globulin Content during Germination.

Rice seeds were soaked in 200 ml of the cycloheximide solution. 100 seeds of each germinating stage were used for analysis.

○—○, control; △—△, 10 $\mu\text{g}/\text{ml}$ cycloheximide and ▲—▲, 20 $\mu\text{g}/\text{ml}$ cycloheximide.

FIG. 4. Change in Glutelin Content during Germination.

Rice seeds were soaked in 200 ml of the cycloheximide solution. 100 seeds of each germinating stage were used for analysis.

○—○, control and △—△ 10 $\mu\text{g}/\text{ml}$ cycloheximide.

degraded even at the 14th day of germination. Horiguchi *et al.* reported the changes of nitrogen compounds of germinating rice seeds.¹⁴⁾ The change of glutelin content of the control in this experiment was similar to their pattern. The breakdown of glutelin was sensitive to cycloheximide and levelled off at about 70% of the control. This result suggests that the endosperm glutelin is degraded partly by the newly synthesized protease. Bechtel and Pomeranz demonstrated that the subaleurone layer of rice seed contained three types of protein bodies: large spherical, small spherical and crystalline protein bodies.¹⁵⁾ Tanaka *et al.* also observed two types of protein bodies: one was spherical with a lamellar structure (PB-I), while the other was stained homogeneously by osmium tetroxide and not of a lamellar structure (PB-II).¹⁶⁾ Tanaka *et al.* observed the undigested residues of rice protein bodies in feces and that the core protein after pepsin digestion was poor in lysine.^{17,18)} On the basis of these findings, it seems likely that there are some differences among protein components of these protein bodies. The fact that about 40% of the

total glutelin remained at the 14th day of germination suggests that there are two types of glutelin: an easily-digested type and a hard one. We have found by electron microscopic studies that the digestibilities of these two types of protein bodies are different in germination. The results will be published elsewhere. Anyway, the 14th day of germination is a turning point in nutrition because rice plants that depend on reserve materials in seeds then require other nutrients. This means that the glutelin remaining at this point will not be used thereafter. Furthermore, whether or not the differences in the utilization and in digestibilities are related to the types of protein bodies remains to be cleared up. Hereafter, more detailed investigations of reserve proteins are necessary to clarify the breakdown mechanisms, related to the proteolytic enzymes and to the physiological roles.

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