Biological Properties of Gibberellin A₁₈ in Comparison with those of Gibberellin A₃

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Abstract

Biological properties of GA₁₈ were compared with those of GA₃ in six different assay systems. GA₁₈ was active in leaf sheath elongation of rice, and d₁ & d₅ mutants of maize, and epicotyl elongation of dwarf pea. The degree of the activity of GA₁₈ was less than 1/10 of that of GA₃ in rice and dwarf pea. In d₁ and d₅ mutants of maize, GA₁₈ was as active as GA₃ at the dosages of 0.1 and 1 μ g per plant. But the activity of GA₁₈ levelled off at 1 μ g per plant. GA₁₈ also stimulated α -amylase activity in the endosperm of rice. However, longer incubation periods were required for the activity of GA₁₈ to be observed than for GA₃. GA₁₈ was inactive in hypocotyl elongation of cucumber and lettuce. The results are discussed in relation to gibberellin biosynthesis.

At present 23 different gibberellins have been isolated and chemically identified^{1,2}. Comparative activities of gibberellins A₁ to A₉ in several assay systems have been studied by a number of investigators (see 3). However, there are only a few reports on the biological activities of other gibberellins^{4,5,6}. The present paper reports on the biological properties of gibberellin A₁₈ (GA₁₈), which was isolated from immature seeds of *Lupinus luteus*^{7,8}, in six different assays in comparison with those of gibberellin A₃ (GA₃).

Materials and Methods

The rice seedling assay: The method followed the description by Ogawa⁹. Seven germinating seeds of rice (a dwarf cultivar, Kotaketamanishiki) were placed in a

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glass tube containing 1 ml of test solutions and covered with a glass wool sheet. The seedlings were grown under fluorescent light (ca. 1,500 lux) at 30°. The length of the second leaf sheath was measured on the 7th day.

The dwarf maize assay: The method followed the description by Katsumi¹⁰⁾. About 7-day-old dwarf seedlings were transplanted to plastic trays and water-cultured under continuous fluorescent light (2,500 lux) at 25°. When the first leaf blade formed a cup-shape, each seedling was treated with a 0.1 ml aliquot of gibberellin solutions¹¹⁾. As a solvent 50% acetone was used. Ten days after treatment the lengths of the first and second leaf sheaths were measured and summed.

The dwarf pea assay: The method of Hayashi and Rappaport¹²⁾ was employed with certain modifications. Seeds of Progress No. 9 were germinated in vermiculite at 25° in the dark. Seedlings with stem lengths of 2.50 to 3.50 cm were selected and transferred to plastic trays. These were water-cultured under red light. When the plants became green and the apical hook was straight, they were treated with gibberellins. Treatment consisted of application of a 0.001 ml aliquot of 90% ethanol solutions of gibberellins to the apical bud of each plant with a microsyringe. After a week the length of the epicotyl was measured.

The cucumber hypocotyl assay: According to the method of Katsumi et al¹³⁾, the activity of gibberellin was determined by measuring the length of the hypocotyl unit of light-grown cucumber seedlings (cv. National Pickling) three days after treatment. Gibberellin was applied to the apical bud of each seedling in a 0.01 ml solution of 95% ethanol.

The lettuce hypocotyl assay: The method of Frankland and Wareing¹⁴⁾ was used. Seeds of lettuce (cv. Grand Rapids) were planted on moist filter paper in the dark at 25°. After two days seedlings with radicles of uniform length (6 mm) were transferred to small petri dishes lined with filter paper wetted with gibberellin solutions. The seedlings were grown under continuous fluorescent light (4,500 lux) at 28°. After 5 days the length of the hypocotyl was measured.

The rice endosperm assay: The method of Ogawa¹⁵⁾ was used. Five sterilized embryoless rice endosperms (cv. Kotaketamanishiki) were placed in a test tube containing test solutions, and incubated at 30°. After 2 days incubation the ambient solution in each test tube was filled up to 5 ml with distilled water and 1 ml of the supernatant fluid was assayed for reducing sugars released from the endosperms by the Somogyi-Nelson colorimetric method. The optical density of the reaction mixture was measured with a spectrophotometer at 590 m μ . The activity of α -amylase in the endosperm was simultaneously examined. The endosperms incubated for 2 or 4 days were ground in a glass mortar, extracted with 5 ml of 0.5% calcium chloride solution and filtered. The filtrate was examined for its α -amylase activity. An 1 ml aliquot of the filtrate was added to 4 ml of 0.25% soluble starch solution of pH 4.8, and incubated at 40°. After 30 minutes, 0.5 ml of the reaction mixture was pipetted to a test tube containing 5 ml of 0.5 M acetic acid and 5 ml of 3×10^{-5} iodine, and the optical density of the mixture was read at 590 m μ . α -Amylase activity was expressed in per cent over the water control.

Results

Leaf sheath elongation: The result of the rice seedling assay is shown in Fig. 1. The activity of GA_{18} was lower than that of GA_3 . No parallel concentration-response curves were obtained for the two gibberellins in the concentration range from 0.001–100 mg/l. As the concentration became higher, the relative activity of GA_{18} to GA_3 was less, the maximum activity of GA_{18} being 10 mg/l. At 100 mg/l there was a slight decrease in activity of GA_{18} . Essentially the same result was also obtained with a different cultivar, Tanginbozu.

In the dwarf maize assay two kinds of mutant, d_1 and d_5 were used. The results are shown in Fig. 2. At dosages of 0.1 and 1 μ g per plant, the activity of GA_{18} was only slightly less active than that of GA_3 in d_1 mutants of maize, but it was the same as GA_3 in d_5 mutants. Being different from GA_3 , the activity of GA_{18} levelled off at 1 μ g per plant in both d_1 and d_5 .

Epicotyl elongation: The activity of GA_3 and GA_{18} in epicotyl elongation of dwarf pea was compared at three dosages. As shown in Fig. 3, the relative activity of GA_{18} was 1/10 of that of GA_3 .

Hypocotyl elongation: The results with the cucumber and lettuce hypocotyl assays are presented in Tables 1 and 2, respectively. GA_{18} was inactive in cucumber hypocotyl elongation at dosages of 0.1, 1 and 10 μ g per plant. It was also inactive in lettuce hypocotyl elongation at the concentrations tested.

Fig. 1. Effect of GA_3 and GA_{18} on the leaf sheath elongation of rice. Measurements were made 7 days after gibberellin treatment. Each point represents the mean of 7 different plants. Standard errors are indicated by vertical lines.

Release of reducing sugars and α -amylase activity: The effect of GA_3 and GA_{18} on the release of reducing sugars from the endosperm and α -amylase activity in the endosperm of rice was tested. As shown in Fig. 4, GA_{18} had no effect on the release

Fig. 2. Effect of GA_3 and GA_{18} on the leaf sheath elongation of d_1 and d_5 mutants of maize. Measurements were made 10 days after gibberellin treatment. Each bar represents the mean of 10 different plants. Standard errors are indicated by vertical lines.

Fig. 3. Effect of GA_3 and GA_{18} on the epicotyl elongation of dwarf pea. Measurements were made 7 days after treatment. Each bar represents the mean of 10 different plants. Standard errors are indicated by vertical lines.

Table 1. Effect of GA₃ and GA₁₈ on the hypocotyl elongation of light-grown cucumber seedlings. Measurements were made 3 days after treatment. Each value (mm) represents the mean of 10 hypocotyl units. Standard errors indicated.

Gibberellin	Dosage, $\mu\text{g}/\text{plant}$			
	0	0.1	1	10
A ₃	23.2 $\pm$ 0.44	25.0 $\pm$ 0.35	32.1 $\pm$ 0.68	45.2 $\pm$ 3.57
A ₁₈	22.7 $\pm$ 0.21	23.3 $\pm$ 0.27	23.2 $\pm$ 0.61	23.3 $\pm$ 0.37

Table 2. Effect of GA₃ and GA₁₈ on the hypocotyl elongation of *Lactuca sativa*, 'Grand Rapids'. Measurements were made 3 days after treatment. Each value (mm) represents the mean of 12-15 different plants. Standard errors indicated.

Gibberellin	Concentration, mg/l				
	0	0.1	1	10	100
A ₃	5.57 $\pm$ 0.20	7.34 $\pm$ 0.53	8.61 $\pm$ 0.52	9.30 $\pm$ 0.92	8.71 $\pm$ 0.56
A ₁₈	5.57 $\pm$ 0.20	4.37 $\pm$ 0.33	4.70 $\pm$ 0.28	4.26 $\pm$ 0.16	4.80 $\pm$ 0.13

Fig. 4. Effect of GA₃ and GA₁₈ on the release of reducing sugars from the endosperm (broken line) and on α -amylase activity in the endosperm of rice (solid line). Each point represents the mean of 3 different test tubes. (a) 2 days incubation (b) 4 days incubation.

of reducing sugars and α -amylase activity when measured after 2 days incubation. On the other hand, when measured after 4 days incubation, GA₁₈ showed a stimulating effect on α -amylase activity (release of reducing sugars was not determined). However, the activity of GA₁₈ was less than 1/10 of that of GA₃.

Discussion

Non-lactonic gibberellins are generally less active than GA₃. According to Brian et al.⁴⁾, gibberellins A₁₂, A₁₃ and A₁₄ are either inactive or at most 1/10 as active as GA₃ depending on the kind of the assay. The activity of GA₂₀ has been reported⁵⁾ to be less than that of GA₃ in the leaf sheath elongation of rice and d₁ mutant of maize, and the hypocotyl elongation of cucumber and stem elongation of dwarf pea. But it is as active as GA₃ in the d₂ and d₅ mutants of maize. The present results also indicate that a non-lactonic gibberellin GA₁₈ is generally less active than GA₃ in six different assays. The lack of activity of GA₁₈ in cucumber and lettuce hypocotyl elongation is probably related to the presence of a hydroxyl group at the C-7 position. In cucumber hypocotyl elongation 7-deoxygibberellins (e.g. A₄, A₇ and A₉) have high activity and in lettuce hypocotyl elongation the presence of a C-7 hydroxyl group is generally disadvantageous⁴⁾.

Some of the non-lactonic gibberellins have been clearly shown to be intermediates of GA₃ biosynthesis. Cross and Norton¹⁶⁾ have demonstrated that GA₁₄ is converted to GA₃ by *Fusarium moniliforme*. The structural difference between GA₁₈ and GA₁₄ is that the former has an extra hydroxyl group at the C-7 position. Introduction of a hydroxyl group at the C-7 position seems to occur after the formation of the lactonic ring in the fungal pathway of GA₃ biosynthesis¹⁷⁾. On the other hand, in higher plants this seems to occur at the earlier step of GA₃ biosynthesis¹⁸⁾. That the occurrence of C-7 hydroxylated non-lactonic gibberellins is restricted to higher plants may support this idea. Therefore, it is possible to assume that GA₁₈ is an intermediate of GA₃ biosynthesis in higher plants. In this connection the present results with d₁ and d₅ mutants of maize are interesting. The levelling-off of the activity of GA₁₈ at 1 μ g per plant may be interpreted as indicating that the rate of conversion of GA₁₈ to a physiologically active gibberellin (e.g., GA₃) is limited in these mutants of maize. The results with the rice endosperm assay (Fig. 5) may also be taken as an evidence that GA₁₈ is converted to other gibberellins. That it takes four days incubation for GA₁₈ to stimulate α -amylase activity may reflect the time lag of the conversion of GA₁₈ to a physiologically active gibberellin. Recently Jones¹⁹⁾ reported that in the barley endosperm assay GA₁₄ requires longer incubation periods for its activity to be observed than does GA₃. He also pointed out the possibility of the conversion of GA₁₄ to a physiologically active gibberellin.

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摘 要

勝見允行*・小川幸持**・小清水弘一***： ジベレリン A_{18} と
ジベレリン A_3 との生物活性の比較

6種類の検定法を用いて、 GA_{18} と GA_3 との生物活性を比較した。 GA_{18} の矮性イネ（小丈玉錦）の葉鞘と矮性エンドウ（Progress No. 9）の茎に対する伸長促進効果は GA_3 の 1/10 以下であった。矮性トウモロコシ d_1 および d_5 の葉鞘伸長促進効果は、0.1 と 1 $\mu\text{g/plant}$ で GA_3 と GA_{18} とは同程度であったが、 GA_{18} の活性は 1 $\mu\text{g/plant}$ で level off した。 GA_{18} はまたイネの胚乳からの還元糖の放出と、胚乳中の α -アミラーゼの活性を促進した。しかし GA_{18} の活性がみとめられるには、 GA_3 よりも長い処理期間を必要とした。 GA_{18} はキウリとレタスの下胚軸に対して伸長効果を示さなかった。これらの諸結果を、 GA_{18} が GA_3 の前駆体であるという見解と関連づけて論議した。（*国際基督教大学生物学教室・**三重大学農学部・***京都大学農学部）