BIOSYNTHESIS OF AVENIC ACID A, A FERRIC CHELATING SUBSTANCE SECRETED FROM AVENA SATIVA L.

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The biosynthesis of avenic acid A was studied by feeding L-methionine-1,3,3,3,4,4,4-d_4 to iron-deficient oat roots (Avena sativa L. cv Amuri II). Both avenic acid A and 2'-deoxymugineic acid labeled with 13C or 2H were isolated from root-washings of the same plants. The 13C-NMR elucidated that three molecules of L-methionine were incorporated into avenic acid A in the same way as 2'-deoxymugineic acid. The 2H-NMR spectra indicated that all deuteriums from the methionine were incorporated into 2'-deoxymugineic acid, but one of the deuteriums at C-4 was lost in avenic acid A. A possible biosynthetic pathway is L-methionine→→2'-deoxymugineic acid→avenic acid A.

KEYWORDS 2'-deoxymugineic acid; avenic acid A; biosynthesis; Avena sativa; L-methionine; 2H-NMR; 13C-NMR

It has been known that gramineous plants respond to iron stress by secreting iron chelating substances which have been generically termed "mugineic acids (MAs)". These substances play a role in solubilization and transport of Fe(III). One of them, avenic acid A (1), was secreted from iron-deficient oat roots (Avena sativa L.).

Since mugineic acid (3) was discovered in barley plants and its structure was first determined, the biosynthesis of MAs has been explored. Kawai et al. fed four 14C-labeled amino acids and L-methionine-1,13C to iron-deficient barley roots, and found that L-methionine is the most efficient precursor for mugineic acid (3) and 2'-deoxymugineic acid (2). Shojima et al. also got the same results in vitro. However, no experimental data on the biosynthesis of avenic acid A (1), or on its relation with other MAs, have been published. In the present study, we found that the precursor for avenic acid A (1) is the same as that for mugineic acid (3) and 2'-deoxymugineic acid (2). A possible biosynthetic pathway for avenic acid A (1) is suggested by the 2H-NMR study.

Eighty 8-day-old oat plants (Avena sativa L. cv Amuri II) were cultured in 3-liter pots containing continuously aerated 1/5 strength Hoagland solution in an environmental chamber. The solution was adjusted to pH 6.0 and renewed once every two days. After 2 weeks, the plants were transferred to a solution free of iron. When the chlorosis developed moderately, 100 μM L-methionine-1,13C or 200 μM D,L-methionine-3,3,3,4,4-
d₄ were fed to the roots with 40 ppm surface-active agent “Decaglyn 1-L” at the end of the secretion. Root-washings were collected the following day by soaking the roots in distilled water from 3 to 5 hour after onset of the light period. The isolation was performed by various chromatographies.³ As a result, ¹³C-enriched avenic acid A (1) (10.8 mg) and 2'-deoxymugineic acid (2) (1.25 mg) and ²H-enriched corresponding compounds (1, 1.8 mg; 2, 0.5 mg) were obtained. The ¹H-, ²H-, and ¹³C-NMR spectra were measured at 500, 76.8, and 75.5 MHz spectrometers, respectively. The assignments were accomplished by ¹H-¹H and ¹³C-¹H COSY, HMBC, and HMQC experiments.

The ¹³C-NMR spectrum showed that labeled avenic acid A (1) (Fig. 1A) was ¹³C-enriched by 11.2, 11.7, and 12.7-fold in their peak-heights for C-1, 4', and 4'', respectively, compared to those of unlabeled avenic acid A (1) (Fig. 1B). This result revealed that three molecules of L-methionine were incorporated into avenic acid A (1) in the same way as mugineic acid (3) and 2'-deoxymugineic acid (2).⁴ The precursor for these different MAs is thought to be the same.

![Fig. 1. ¹³C-NMR Spectra of Avenic Acid A (1) Biosynthesized from L-Methionine-1-¹³C (B) and Unlabeled Avenic Acid A (1) (A)](image)

Avenic acid A (1) and 2'-deoxymugineic acid (2) were simultaneously isolated from the same plants; however, their relationship in the biosynthetic pathway is not clear. It is helpful, therefore, to know how protons in methionine change during the biosynthesis to avenic acid A (1) and 2'-deoxymugineic acid (2). To investigate this, both ²H-enriched avenic acid A (1) and 2'-deoxymugineic acid (2) were isolated, and their ²H-NMR was performed. As ²H chemical shifts correspond to ¹H shifts aside from a small isotope effect and broadening, we can speculate the assignment of ²H-NMR peaks based on that of ¹H-NMR. As shown in Fig. 2B, relative peak intensity at δ 4.12, 4.01, 3.51, 3.39, 3.22, 2.77, 2.57, 2.18, 2.06 in labeled 2'-deoxymugineic acid (2) was 1:1:1:2:1:1:3:1, corresponding deuteriums at C-4, C-1', C-1'', C-3, C-2', and C-2'', respectively. This result suggests that 12 deuteriums from three molecules of methionine-3,3,4,4-d₄ were wholly incorporated. In comparison, 11 deuteriums whose relative peak intensity at δ 3.60, 2.58, 1.76 was 1:4:6 were observed in the labeled avenic acid A (1) (Fig. 3B). One of the deuteriums at C-4 was lost in the labeled avenic acid A (1), but all other deuteriums were incorporated in the same way as the labeled 2'-deoxymugineic acid (2). These results suggest that avenic acid A (1) can not be the precursor of 2'-deoxymugineic acid (2). However, the reverse may be possible—that is, avenic acid A (1) is biosynthesized from 2'-deoxymugineic acid (2) by cleavage of azetidine ring. A possible biosynthetic pathway for avenic acid
A (1) may be suggested as \( \text{L-methionine} \rightarrow 2'-\text{deoxymugineic acid (2)} \rightarrow \text{avenic acid A (1)}. \)

Fig. 2. \( ^1\text{H-NMR Spectrum of 2'}-\text{Deoxymugineic Acid (2) (A) and 2H-NMR Spectrum of 2'}-\text{Deoxymugineic Acid (2) Biosynthesized from D,L-Methionine-3,3,4,4-d}_4 \) (B).

The peak at \( \delta 4.93 \) represents the signal of water.

Fig. 3. \( ^1\text{H-NMR Spectrum of Avenic Acid A (1) (A) and 2H-NMR Spectrum of Avenic Acid A (1) Biosynthesized from D,L-Methionine-3,3,4,4-d}_4 \) (B).

The peak at \( \delta 4.80 \) represents the signal of water.

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**REFERENCES**


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