Shinobu Satoh

University of Tsukuba, Institute of Biological Sciences

1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan

e-mail : satoh.shinobu.ga@u.tsukuba.ac.jp

The subject area:

(5) Physiology/Biochemistry/Molecular and Cellular Biology

Number of tables, black-and-white figures, and color figures:

6 black-and-white figures

Promotion of auxin- and gibberellin-induced elongation of epicotyl segments of *Vigna angularis* by short-chain carboxylic acids

Shinobu Satoh[1), 2)]

1) University of Tsukuba, Institute of Biological Sciences (present affiliation)

1-1-1 Tennodai, Tsukuba, Ibaraki 305-8572, Japan

2) Yokohama City University, Biological Institute (affiliation where and when the experiments were performed)

22-2 Seto, Kanazawa-ku, Yokohama 236-0027, Japan

e-mail: satoh.shinobu.ga@u.tsukuba.ac.jp
Tel: 029-853-4672

Abstract

Pollen tube growth is inhibited and promoted by long- and short-chain carboxylic acids, respectively, but is not affected by formic acid. For auxin- and gibberellin-induced elongation of *in vitro* cultured epicotyl segments of adzuki bean (*Vigna angularis*), a series of carboxylic acids showed similar effects as that on pollen tube growth except that formic acid showed the strongest promotive effect. The effects of formic acid and GA_3 on IAA-induced elongation were additive and both were strongly inhibited by inhibitors of cellulose synthesis (coumarin) and microtubule formation (colchicine). Formic acid, possibly by incorporation into the segments, prolonged the promotion by IAA and GA_3 of the elongation of epicotyl segments. Based on these results and later advances in our understanding of metabolism and the role of formic acid in protecting against oxidative stress, a possible role of formic acid on stem elongation is discussed.

Key Words

Auxin, Carboxylic acid, Cell wall, Elongation, Epicotyl, Formic acid, Gibberellin, Oxidative stress

Abbreviations

GA_3, gibberellin A3; IAA, indole-3-acetic acid; ROS, reactive oxygen species

Introduction

Some ants collect insect larvae, plant seeds, and fungus spores in their nests. Myrmicacin (1-3-hydroxydecanoic acid) has been isolated from the secretions of one such species, [A1] the South American leaf-cutting ants (*Atta sexdens*). Myrmicacin inhibits the germination of *Alternaria* and *Botrytis* spores (Schildknecht and Koob 1971). It was assumed that ants apply myrmicacin-containing secretions to seeds or spores to prevent their germination. Iwanami and Iwadare (1978) reported that myrmicacin inhibits the germination, tube elongation, and tube mitosis of the pollen grains of several higher plant species. Furthermore, they reported the effects of a variety of fatty acids and their analogous compounds on pollen tube growth and mitosis (Iwanami 1980; Iwanami and Iwadare 1979). The results are summarized as follows. A carboxyl group is indispensable for the inhibitory effects. Long-chain carboxylic acids are more effective than short-chain carboxylic acids. Carboxylic acids with a cyclic carbon chain have the same inhibitory effects as straight-chain carboxylic acids. Compounds containing a π electron system or polar group near the carboxyl group have inhibitory activity, but compounds containing these groups distant from the carboxyl group do not. Dicarboxylic acids have no inhibitory activity but at low concentrations promote tube growth.

In 1979, I examined the effects of a series of carboxylic acids (C_1 to C_{10}) on the pollen-tube growth and the auxin- and gibberellin-induced growth of stem segments with Iwanami to assess the effect of carbon-chain length on plant cell growth, of which there are two types—tip and diffuse growth (Kropf *et al*. 1998). Pollen-tube growth, a type of tip growth, occurs by adding tube wall substances to the tip of the pollen tube. Diffuse growth occurs in the cell wall of stem cells.

The effects of carboxylic acids on the pollen-tube growth of *Camellia japonica* were published by Iwanami and Satoh (1980) and are summarized as follows. Capric acid (C_{10}) and caprylic acid (C_8) inhibited pollen-tube elongation even at low concentrations (25 ppm). Enanthic acid (C_7), caproic acid (C_6), valeric acid (C_5), and butyric acid (C_4) had inhibitory activity only at higher concentrations (50 to 100 ppm) but promoted pollen-tube elongation at lower concentrations

(below 25 ppm). Propionic acid (C_3) and acetic acid (C_2) had promoting activity at a wide range of concentrations but had no inhibitory activity. Formic acid (C_1) had no activity.

Thereafter, formic and acetic acids were reported to be present in acid rain (Keen *et al*. 1983), and pyroligneous acid, a byproduct of charcoal production that contains high levels of organic acids such as acetic acid and formic acid (Ménard *et al*. 1984), is used as a growth regulator in agriculture (Grewal *et al*. 2018). Indeed, application of formic acid reportedly promotes the growth of rice plants (Shiraishi *et al*. 2000a). Acetic acid produced in response to dry stress is involved in drought tolerance in Arabidopsis and application of acetic acid enhances the dry tolerance of a variety of plant species (Kim *et al*. 2017). Therefore, I analyzed stored data on the effects of carboxylic acids on the growth of stem (epicotyl) segments to gain insight into the mechanism controlling cell growth.

Stem elongation in the region below the apical meristem where cell division has ceased is promoted by the actions of auxin and gibberellin. Auxin promotes cell elongation by promoting cell-wall loosening via the activation of cell wall enzymes and proteins. This activation is mediated by a drop in pH caused by activation of plasma membrane H^+-ATPase (Arsuffi and Braybrook 2018). *De novo* cellulose synthesis was not required for auxin-induced growth *in vitro* of epicotyl segments of adzuki bean (Hogetsu *et al*. 1974). However, gibberellin promoted cell elongation by orienting cellulose microfibrils under the direction of cortical microtubules, which was accompanied by *de novo* cellulose synthesis, in epicotyl segments (Shibaoka 1994).

Here, I analyzed the effects of a series of carboxylic acids on auxin- and gibberellin-induced stem elongation, and the effects of pH and of inhibitors of cellulose synthesis (coumarin) and microtubule formation (colchicine) on the actions of formic acid using *in vitro*-cultured epicotyl segments of adzuki bean (*Vigna angularis* (Willd.) Ohwi & H.Ohashi).

Materials and Methods

The effects of carboxylic acids on auxin- and gibberellin-induced elongation of epicotyl segments

were assayed according to the method of Shibaoka (1972). Seedlings of adzuki bean (*V. angularis*) grown with vermiculite under white fluorescent light (20.5 μmol m^{-2} s^{-1}) at 25°C for 6 days were used. A 10-mm segment was obtained from the seedling by cutting the epicotyl at 5 and 15 mm below the node of the first leaves using a double-bladed cutting tool. For each test, ten segments were incubated in a flat dish (diameter 60 mm) with a lid under fluorescent light (20.5 μmol m^{-2} s^{-1}) at 25 °C for 18 h. The dishes were filled with 3 mL of test solution, composed of basal medium (1/60 M potassium phosphate buffer [pH 6.2] containing 2% sucrose without or with 1×10^{-4} M indole-3-acetic acid [IAA] or 1×10^{-4} M IAA + 1×10^{-4} M gibberellin A3 [GA_3]), to which the test substances were added. After incubation, the length of each segment was measured using a small projector. The substances used in this study were C_1, formic acid (COOH); C_2, acetic acid (C_1-COOH); C_4, butyric acid (C_3-COOH); C_6, caproic acid (C_5-COOH); C_8, caprylic acid (C_7-COOH); and C_{10}, capric acid (C_9-COOH).

To evaluate the effect of the concentration of formic acid, the pH of the test solution was adjusted to 5.0 by titration with KOH and the change in pH was recorded after incubation for 18 h. To assess the effect of pH on the effects of formic acid, the pH of the test solution was adjusted by titration with KOH or HCl and the change in pH was recorded after incubation for 18 h. To evaluate the effects of inhibitors of cellulose synthesis and microtubule formation, 5×10^{-4} M coumarin and 1×10^{-3} M colchicine were added to the test solution according to Hogetsu *et al*. (1974) and Shibaoka (1972), respectively.

Results

Fig. 1a–f shows the effects of carboxylic acids on the elongation of adzuki bean epicotyl segments. Capric acid (C_{10}) did not affect IAA- and GA_3-induced elongation as well as that in the absence of hormones (Fig. 1a). Caprylic acid (C_8) and caproic acid (C_6) at higher concentrations showed slight inhibition and promotion of IAA- and GA_3-induced elongation, respectively (Fig. 1b, c). Butyric acid (C_4), acetic acid (C_2), and formic acid (C_1) showed no inhibition but promoted IAA- and

GA_3-induced elongation; no effect was observed in the absence of hormones (Fig. 1d–f). The magnitude of the promotion increased with decreasing number of carbon atoms in the chain (Fig. 1d–f). However, the promotion effect of formic acid decreased at 240 ppm (Fig. 1f). This may be a result of a decrease in the pH of the test solution, because formic acid is the strongest among monovalent carboxylic acids. In preliminary experiments using basal medium containing potassium phosphate buffer pH 5.0 (versus pH 6.2 in this study), the promotion by caproic acid (C_6) and acetic acid (C_2) was greater than that in pH 6.2 buffer. However, the promotion by higher concentrations of butyric acid (C_4), acetic acid (C_2), and formic acid (C_1) decreased with the pH of the test solutions < 4.0 (data not shown).

Because formic acid (C_1) showed the strongest promoting effect at 120 ppm (2.6 mM) among C_1 to C_{10} carboxylic acids (Fig. 1f), and the pH of the test solution was 5.0, I next examined the effect of formic acid concentration at pH 5.0. As shown in Fig. 2a, the IAA- and GA_3-induced elongation of the segments was promoted by 120 to 240 ppm formic acid; the effect peaked at 180 and 240 ppm. The pH of the culture medium after 18 h increased to almost 6 in accordance with the increase in the formic acid concentration to 120 ppm in the presence or absence of hormones (Fig. 2b).

I next examined the effect of 120 ppm formic acid at various pH values. As shown in Fig. 3a, promotion by 120 ppm formic acid was strongest at pH 4.0, probably due to the balance between the effects of formic acid and hazard by low pH. pH did not influence the elongation of epicotyl segments in the absence of formic acid (Fig. 3c). After incubation for 18 h, the pH of the pH 4.5 and 5.5 culture medium increased by 0.5 to 1.0 units in the presence of formic acid (Fig. 3b). The increase in pH increase was reduced in the absence of formic acid (Fig. 3d).

The changes in segment length [A2] in the test solution containing 120 ppm formic acid at pH 4.0 are shown in Fig. 4. In the absence of formic acid, [A3] the rate of IAA- and GA_3-induced elongation decreased at 8 and 10 h, respectively, but elongation was maintained even after 8 h and 10 h, respectively in the presence of formic acid, whereas addition of GA_3 promoted elongation at the

start of incubation compared with IAA only. The effects of GA_3 and formic acid on IAA-induced elongation were additive (Fig. 4).

Next, to determine the role of cell wall synthesis in the promotion of elongation of segments by formic acid, coumarin (inhibitor of cellulose synthesis) and colchicine (inhibitor of microtubule formation) were used. In the presence of IAA, 5×10^{-4} M coumarin strongly inhibited the promotion by formic acid of IAA-induced elongation but did not affect IAA-induced elongation (Fig. 5a). In the presence of GA_3 and IAA, coumarin strongly inhibited the effects of formic acid and GA_3 (Fig. 5b).

Similarly, 1×10^{-3} M colchicine strongly inhibited the effect of formic acid on IAA- and GA_3-induced elongation, albeit to a lesser degree than coumarin (Fig. 6a, b). Moreover, colchicine completely inhibited the effects of GA_3 but had no effect on IAA-induced elongation (Fig. 6a, b).

Discussion

The tendency of the effects of a series of carboxylic acids on stem elongation was similar to that on pollen-tube elongation with respect to the correlation with carbon-chain length. Longer chain-carboxylic acids showed inhibitory activity at higher concentrations and those with shorter chains showed concentration-dependent promotion in the presence of IAA and GA_3 (Fig. 1). Formic acid (C_1) had no effect on pollen-tube elongation but promoted stem elongation. Formic acid has aldehyde and carboxyl groups, and the reducibility of the aldehyde group may be related to the difference between its effects and those of the other acids.

After incubation for 18 h, the pH of formic-acid-containing culture medium increased to around 6 depending on the concentration of formic acid in the presence and absence of hormones (Fig. 2b). Under acidic conditions, carboxylic acids penetrate the cytosol through the plasma membrane because of the abundance of non-dissociated molecules, and subsequently dissociate/ionize in the alkaline cytosol, reducing its pH (Brummer *et al*. 1984). Therefore, the 0.5 to 1.0-unit increase in pH in culture medium after incubation for 18 h (Fig. 3b) is likely a result of incorporation of formic acid into epicotyl cells. Because pH increased even in the absence of

hormones, formic acid incorporated into cells may not be directly associated with elongation. In contrast, the almost 0.5-unit pH increase after incubation for 18 h at pH 3.5 to 4.5 in the absence of formic acid (Fig. 3d) may be a result of phosphate uptake.

The effects of GA_3 and formic acid on IAA-induced elongation were additive (Fig. 4), and the action sites of GA_3 and formic acid on IAA-induced elongation are different, because GA_3 increased the growth rate at the start of incubation and formic acid after several hours. This suggests that formic acid maintains auxin- and gibberellin-induced stem elongation.

Furthermore, the inhibition by coumarin and colchicine of the promotion by formic acid of IAA- and GA_3-induced elongation (Figs. 5, 6) suggests a relationship between the action of formic acid and cell wall synthesis. Those compounds strongly inhibited GA_3-induced elongation but did not affect IAA-induced elongation, as reported by Hogetsu *et al*. (1974) and Shibaoka (1972). Auxin-induced elongation is known as acid growth and is not accompanied by *de novo* synthesis of cellulose (Arsuffi and Braybrook 2018). The different magnitudes of inhibition of coumarin and colchicine may be related to the direct or indirect effects of inhibitors of cellulose synthesis.

The possible uptake of formic acid into cells was not correlated with the changes in the growth of segments in the presence or absence of hormones (Fig. 3a, b). Therefore, formic acid is not used in cell wall [A4] formation, but it prevents slowdown of IAA- and GA_3-induced elongation.

Regarding the roles of carboxylic acids in cell growth, there are three possibilities. First, short-chain carboxylic acids reduce cytosolic pH (Kurkdjian and Guern 1989). A change in the cytosolic free Ca^{2+} concentration is reportedly associated with the decrease of cytosolic pH induced by IAA (Felle 1988). Therefore, a drop in cytosolic pH could influence second messengers, such as Ca^{+}, triggering a physiological response. Although the effects of exogenously applied formic acid on cytoplasmic pH have not been reported, a possible drop in pH of the cytosol may cause stem elongation [A5] as formic acid is a stronger acid than acetic acid (pKa 3.77 and 4.76, respectively). Second, in Arabidopsis, dry stress induced accumulation of acetic acid to 2 mmol per kg fresh weight, causing histone acetylation and activation of the jasmonic acid signaling pathway. Indeed,

application of 10 to 30 mM acetic acid enhanced drought tolerance in various plant species (Kim *et al*. 2017). [A6] A similar mechanism may be involved in the regulation of stem elongation by acetic acid.

Third, [A7] certain ants make use of formic acid as a toxin or alarm pheromone (Löfqvist 1976). *Urtica* plants use formic acid as a toxin in stinging hairs (Thurston and Lersten 1969). In green tissues of C3 plants, formic acid is produced via the photorespiratory pathway by decarboxylation of glyoxylate under light (Wingler *et al*. 1999). Formic acid is oxidized by formate dehydrogenase into CO_2 (Hanson and Roje 2001). Application of formic acid (2 mM) to rice plants promoted growth and upregulated the transcription of formate dehydrogenase (Shiraishi *et al*. 2000a). Moreover, formic acid prevented inhibition of photosynthesis in rice leaves under high light and low CO_2, possibly by endogenous reactive oxygen species (ROS) scavenging and/or by supplying CO_2 to suppress photorespiration (Shiraishi *et al*. 2000b). The concentration of formic acid was reported to be around 1 μmol g fresh weight^{-1} (≈ 1 mM [46 ppm]) with increases under higher photorespiratory conditions (lower CO_2 partial pressure) in pea (*Pisum sativum*) seedlings (Amory *et al*. 1992). Those reports suggest that formic acid is synthesized in plants to protect cells from oxidative stress and maintain photosynthesis. Because the adzuki bean epicotyl is mainly composed of chloroplast-harboring cells, ROS may be produced during *in vitro* culture of epicotyl segments under light and low CO_2 in the culture medium, resulting in suppression of elongation. This may explain why formic acid did not affect the growth of pollen tubes (which lack chloroplasts) in the absence of light (Iwanami and Satoh 1980). Moreover, oxidative stress may be critical for the function of cortical microtubules in controlling cellulose deposition in the diffuse growth of stem cells; cortical microtubules are not essential for tip growth of the pollen tube (Kropf *et al*. 1998).

I propose the following roles for formic acid in plant growth. Formic acid supplied exogenously or synthesized in epicotyl segments prevents the slowdown of IAA- and GA_3-induced elongation by ameliorating oxidative stress. It is possible that a similar mechanism functions in intact plants under stress, such as high light intensity or drought. Such conditions cause stomatal closure,

restricting the CO_2 supply and promoting photorespiration. Further physiological and molecular biological research is required to evaluate this hypothesis.

Acknowledgements

I thank Prof. Yozo Iwanami of Yokohama City University for his advice as the supervisor of my graduate research at Yokohama City University. I also thank the late Prof. Hiroh Shibaoka of Tokyo Metropolitan University for his technical advice regarding the culture of adzuki bean epicotyl segments.

References

Amory AM, Ford L, Pammenter NW, Cresswell CF (1992) The use of 3-amino-1,2,4-triazole to investigate the short-term effects of oxygen toxicity on carbon assimilation by *Pisum sativum* seedlings. Plant Cell Environ 15: 655-663

Arsuffi G, Braybrook SA (2018) Acid growth: an ongoing trip. J Exp Bot 69: 137–146

Brummer B, Felle H, Parish RW (1984) Evidence that acid solutions induce plant cell elongation by acidifying the cytosol and stimulating the proton pump. FEBS Lett 174: 223–227

Felle H (1988) Auxin causes oscillations of cytosolic free calcium and pH in *Zea mays* coleoptiles. Planta 174: 495–499

Grewal A, Abbey L, Gunupuru LR (2018) Production, prospects and potential application of pyroligneous acid in agriculture. J Anal Appl Pyrolysis 135: 152–159

Hanson AD, Roje S (2001) One-carbon metabolism in higher plants. Annu Rev Plant Physiol Plant Mol Biol 52:119–137

Hogetsu T, Shibaoka H, Shimokoriyama M (1974) Involvement of cellulose synthesis in actions of gibberellin and kinetin on cell expansion. Plant Cell Physiol 15: 265–272

Iwanami Y (1980) Stimulation of pollen tube growth *in vitro* by dicarboxylic acids. Protoplasma 102: 111–115

Iwanami Y, Iwadare T (1978) Inhibiting effects of myrmicacin on pollen growth and pollen tube

mitosis. Bot Gaz 139: 42–45

Iwanami Y, Iwadare T (1979) Myrmic acids: a group of new inhibitors analogous to myrmicacin (β-hydroxydecanoic acid). Bot Gaz 140: 1–4

Iwanami Y, Satoh S (1980) Effects of short chain carboxylic acids on pollen growth. Jpn J Palynol 26: 13–18

Keene WC, Galloway JN, Holden Jr. JD (1983) Measurement of weak organic acidity in precipitation from remote areas of the world. J Geophys Res 88: 5122–5130

Kim JM, To TK, Matsui A, Tanoi K, Kobayashi NI, Matsuda F, Habu Y, Ogawa D, Sakamoto T, Matsunaga S, Bashir K, Rasheed S, Ando M, Takeda H, Kawaura K, Kusano M, Fukushima A, Endo TA, Kuromori T, Ishida J, Morosawa T, Tanaka M, Torii C, Takebayashi Y, Sakakibara H, Ogihara Y, Saito K, Shinozaki K, Devoto A, Seki M (2017) Acetate-mediated novel survival strategy against drought in plants. Nature Plants 3: 17097

Kropf DL, Bisgrove SR, Hable WE (1998) Cytoskeletal control of polar growth in plant cells. Curr Opini Cell Biol 10: 117–122

Kurkdjian A, Guern J (1989) Intracellular pH: measurement and importance in cell activity. Annu Rev Plant Physiol Plant Mol Biol 40: 271–303

Löfqvist J (1976) Formic acid and saturated hydrocarbons as alarm pheromones for the ant *Formica rufa*. J Insect Physiol 22: 1331–1346

Ménard H, Gaboury A, Bélanger D, Roy C (1984) High-performance liquid chromatographic analysis of carboxylic acids in pyroligneous liquors. J Anal Appl Pyrolysis 6: 45–57

Schildknecht H, Koob K (1971) Myrmicacin, the first insect herbicide. Angew Chem Internat Edit 10: 124–125

Shibaoka H (1972) Gibberellin-colchicine interaction in elongation of azuki bean epicotyl sections. Plant Cell Physiol 13: 461–469

Shibaoka H (1994) Plant hormone-induced changes in the orientation of cortical microtubules: alterations in the cross-linking between microtubules and the plasma membrane. Annu Rev Plant

Physiol Plant Mol Biol 45:527–544

Shiraishi T, Fukusaki E, Kobayashi A (2000a) Formate dehydrogenase in rice plant: growth stimulation effect of formate in rice plant. J Biosci Bioeng 89: 241–246

Shiraishi T, Fukusaki E, Miyake C, Yokota A, Kobayashi A (2000b) Formate protects photosynthetic machinery from photoinhibition. J Biosci Bioeng 89: 564–568

Thurston EL, Lersten NR (1969) The morphology and toxicology of plant stinging hairs. Bot Rev 63: 393–412

Wingler A, Lea PJ, Leegood RC (1999) Photorespiratory metabolism of glyoxylate and formate in glycine-accumulating mutants of barley and *Amaranthus edulis*. Planta 207: 518–526

Legends to Figures

Fig. 1. Effects of short-chain carboxylic acids on the elongation of adzuki bean epicotyl segments. Epicotyl segments of 10 mm length were incubated in dishes filled with three types of basal medium without (circle) or with IAA (square) or IAA + GA_3 (triangle) with C_{10}, capric acid (**a**); C_8, caprylic acid (**b**); C_6, caproic acid (**c**); C_4, butyric acid (**d**); C_2, acetic acid (**e**); and C_1, formic acid (**f**). The length of each segment was measured after 18 h. Error bars indicate standard deviations (n = 10).

Fig. 2. Effect of formic acid concentration at pH 5.0. Epicotyl segments were incubated in test solution without (circle) or with IAA (square) or IAA + GA_3 (triangle); pH was adjusted to 5.0, and segment length was measured after 18 h (**a**). Error bars indicate standard deviations (n = 10). pH of culture medium after 18 h (**b**).

Fig. 3. Effect of pH on the effects of formic acid. Epicotyl segments were incubated in test solution without (circle) or with IAA (square) or IAA + GA_3 (triangle) in the presence (**a**, **b**) or absence (**c**, **d**) of 120 ppm formic acid; pH was adjusted as indicated. Segment length was measured after 18 h (**a**, **c**). Error bars indicate standard deviations (n = 10). pH of culture medium after 18 h (**b**, **d**).

Fig. 4. Time course of the elongation of epicotyl segments in the presence of hormones and formic acid. Epicotyl segments were incubated in test solution with (open symbols) or without (closed

symbols) formic acid (120 ppm) in the presence of IAA (square) or IAA + GA_3 (triangle) (pH 4.0). Segment length was measured at the indicated times. Error bars indicate standard deviations (n = 10).

Fig. 5. Effect of coumarin on the elongation of epicotyl segments induced by hormones and formic acid. Epicotyl segments were incubated in test solution with (open symbols) or without (closed symbols) 5×10^{-4} M coumarin in the presence of IAA (a) or IAA + GA_3 (b) (pH 4.0). Segment length was measured at the indicated times. Error bars indicate standard deviations (n = 10). Square, circle, diamond, and triangle indicate IAA, IAA + formic acid, IAA + GA_3, and IAA + GA_3 + formic acid, respectively.

Fig. 6. Effect of colchicine on elongation induced by hormones and formic acid. Epicotyl segments were incubated in test solution with (open symbols) or without (closed symbols) 1×10^{-3} M colchicine in the presence of IAA (**a**) or IAA + GA_3 (**b**) (pH 4.0). Segment length was measured at the indicated times. Error bars indicate standard deviations (n = 10). Square, circle, diamond, and triangle indicate IAA, IAA + formic acid, IAA + GA_3, and IAA + GA_3 + formic acid, respectively.

Fig 1

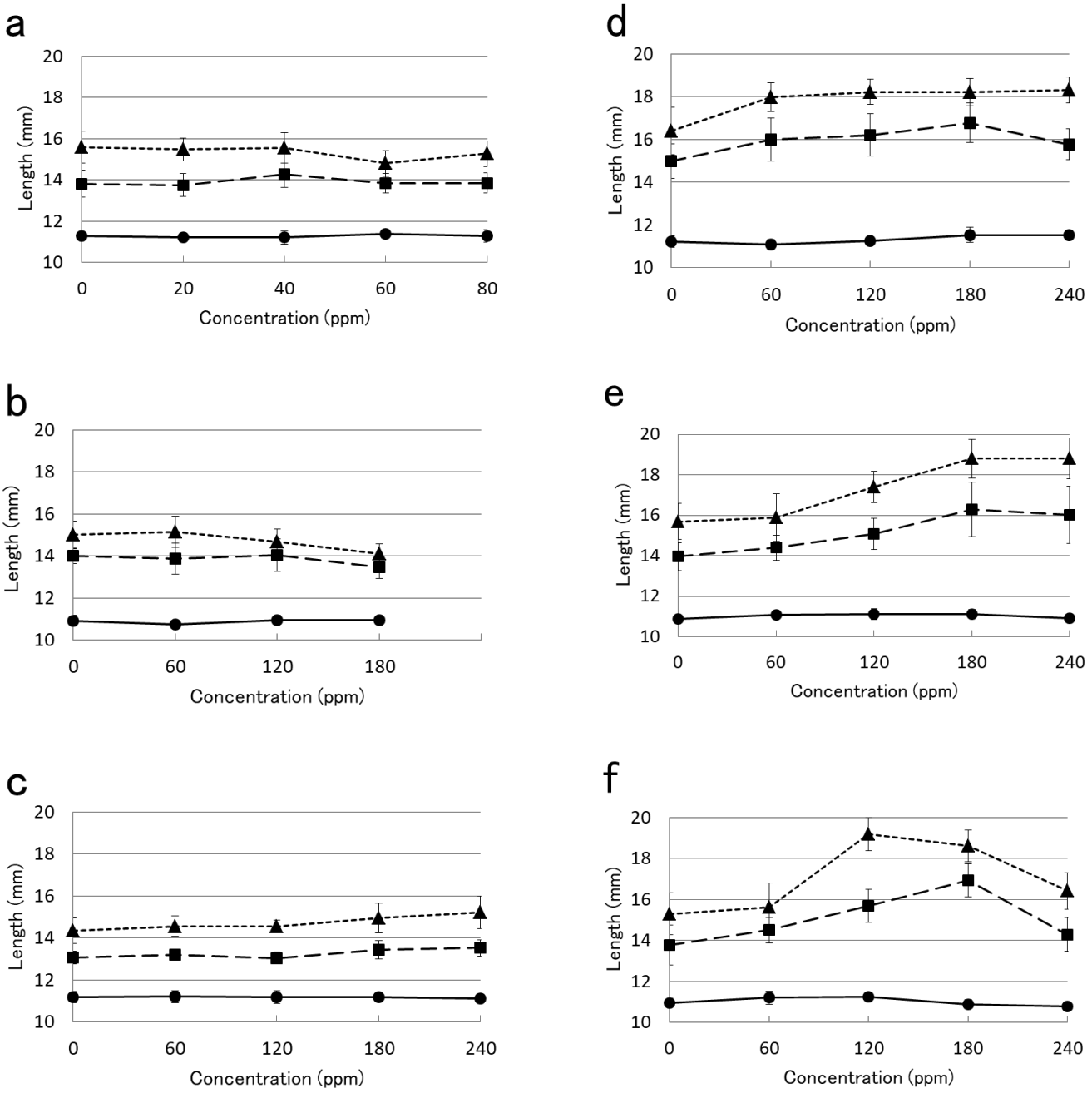

Fig. 1. Effects of short-chain carboxylic acids on the elongation of adzuki bean epicotyl segments. Epicotyl segments of 10 mm length were incubated in dishes filled with three types of basal medium without (circle) or with IAA (square) or IAA + GA_3 (triangle) with C_{10}, capric acid (a); C_8, caprylic acid (b); C_6, caproic acid (c); C_4, butyric acid (d); C_2, acetic acid (e); and C_1, formic acid (f). The length of each segment was measured after 18 h. Error bars indicate standard deviations (n = 10).

Fig 2

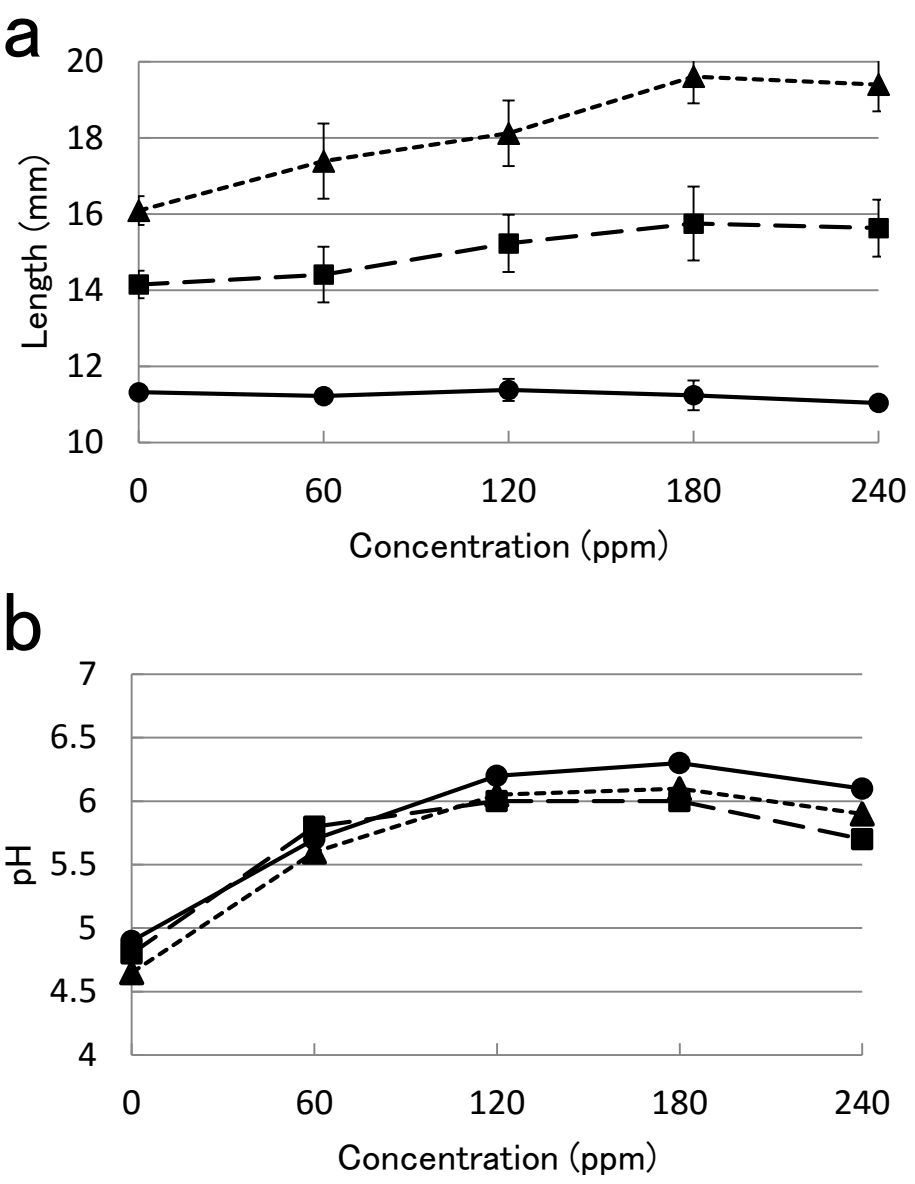

Fig. 2. Effect of formic acid concentration at pH 5.0. Epicotyl segments were incubated in test solution without (circle) or with IAA (square) or IAA + GA_3 (triangle); pH was adjusted to 5.0, and segment length was measured after 18 h (a). Error bars indicate standard deviations (n = 10). pH of culture medium after 18 h (b).

Fig 3

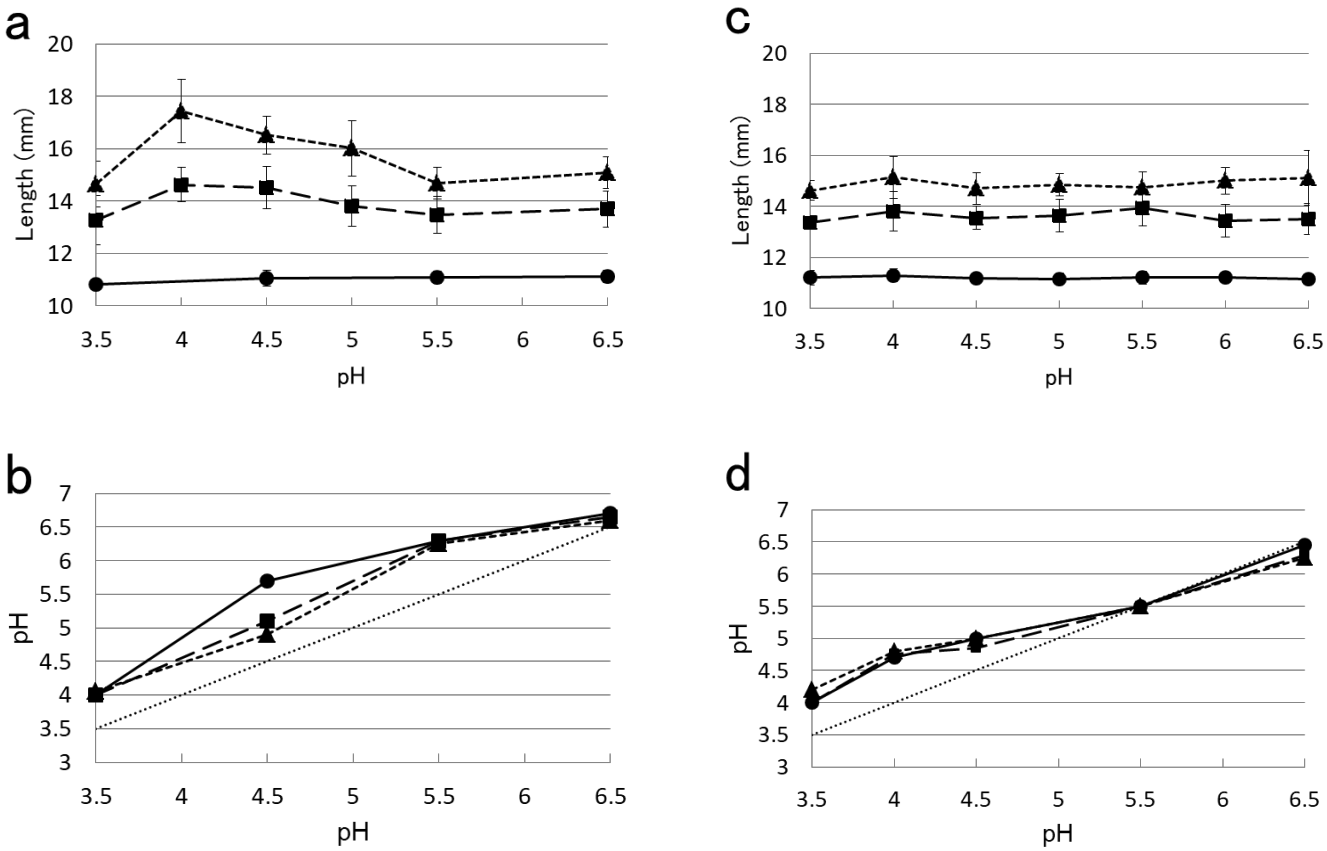

Fig. 3. Effect of pH on the effects of formic acid. Epicotyl segments were incubated in test solution without (circle) or with IAA (square) or IAA + GA_3 (triangle) in the presence (a, b) or absence (c, d) of 120 ppm formic acid; pH was adjusted as indicated. Segment length was measured after 18 h (a, c). Error bars indicate standard deviations (n = 10). pH of culture medium after 18 h (b, d).

Fig 4

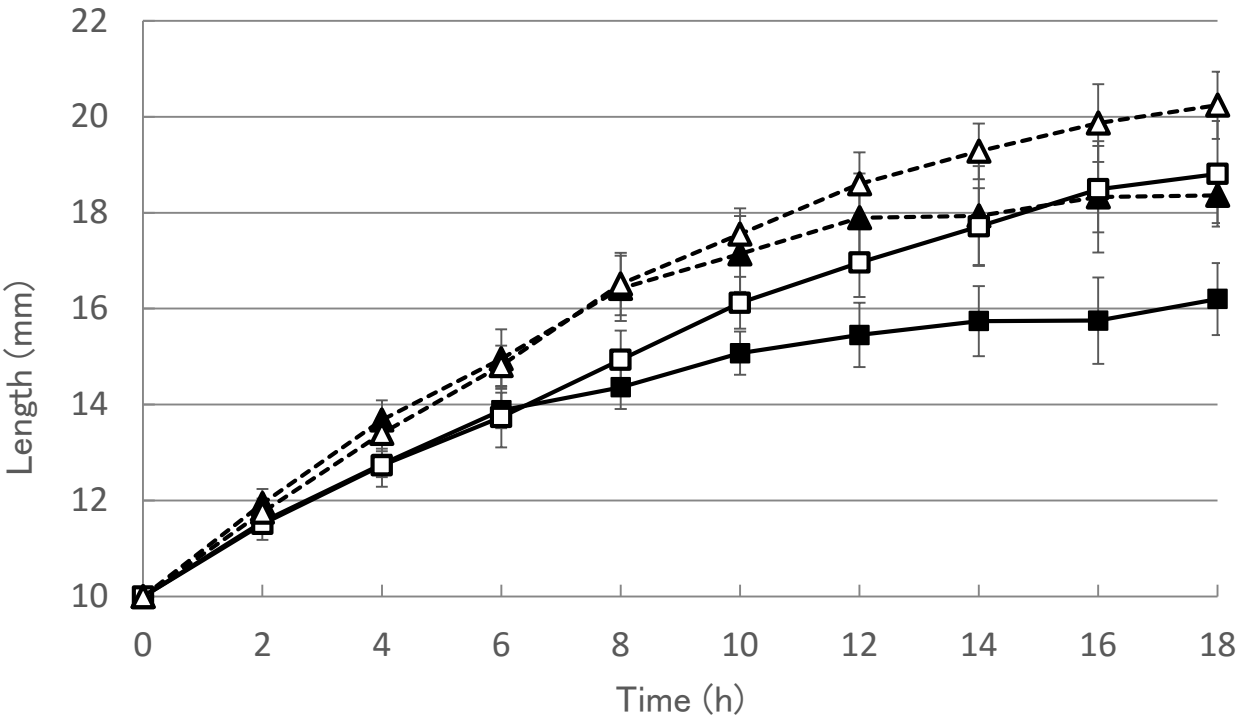

Fig. 4. Time course of the elongation of epicotyl segments in the presence of hormones and formic acid. Epicotyl segments were incubated in test solution with (open symbols) or without (closed symbols) formic acid (120 ppm) in the presence of IAA (square) or IAA + GA_3 (triangle) (pH 4.0). Segment length was measured at the indicated times. Error bars indicate standard deviations (n = 10).

Fig 5

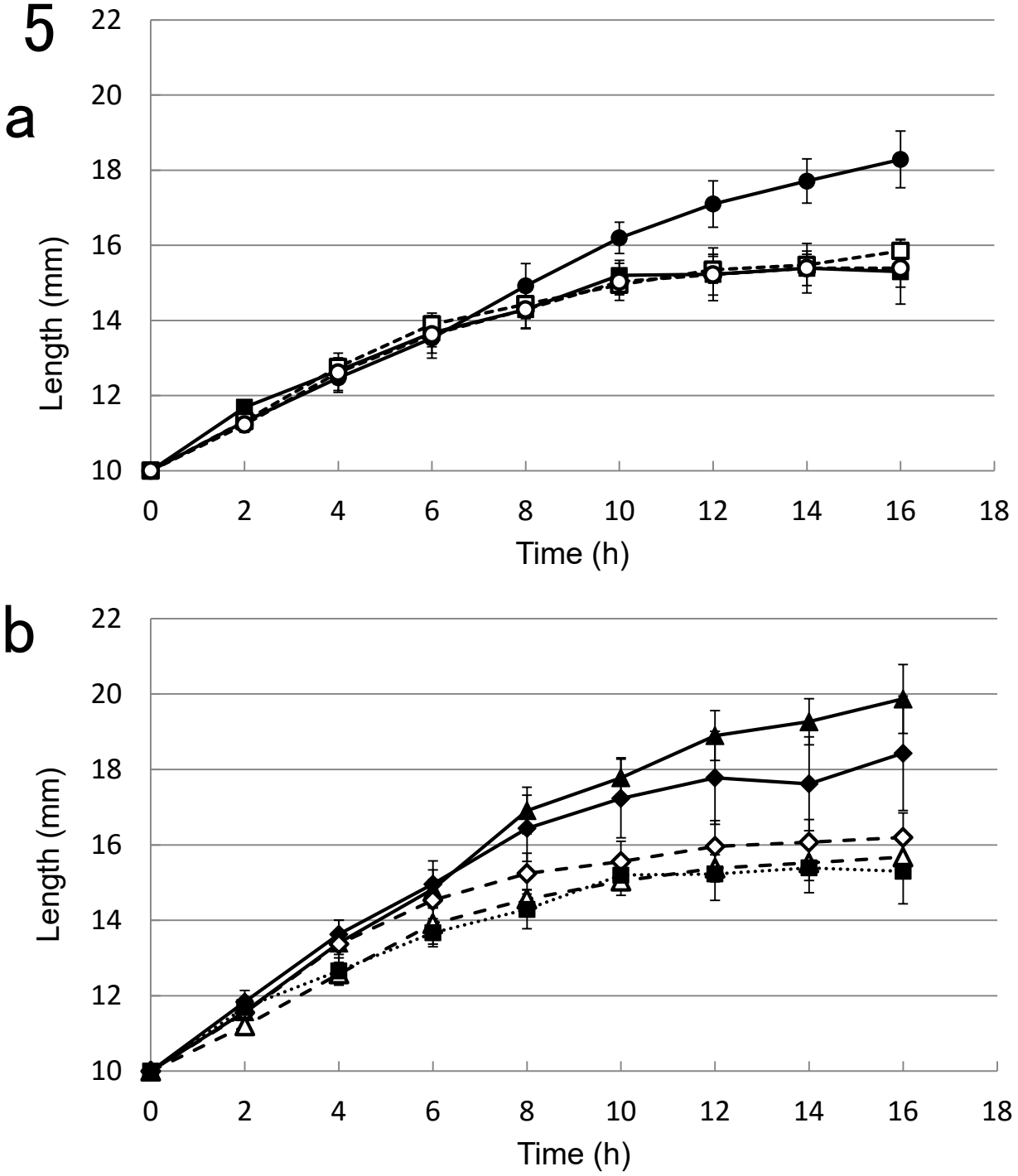

Fig. 5. Effect of coumarin on the elongation of epicotyl segments induced by hormones and formic acid. Epicotyl segments were incubated in test solution with (open symbols) or without (closed symbols) 5×10^{-4} M coumarin in the presence of IAA (a) or IAA + GA_3 (b) (pH 4.0). Segment length was measured at the indicated times. Error bars indicate standard deviations (n = 10). Square, circle, diamond, and triangle indicate IAA, IAA + formic acid, IAA + GA_3, and IAA + GA_3 + formic acid, respectively.

Fig 6

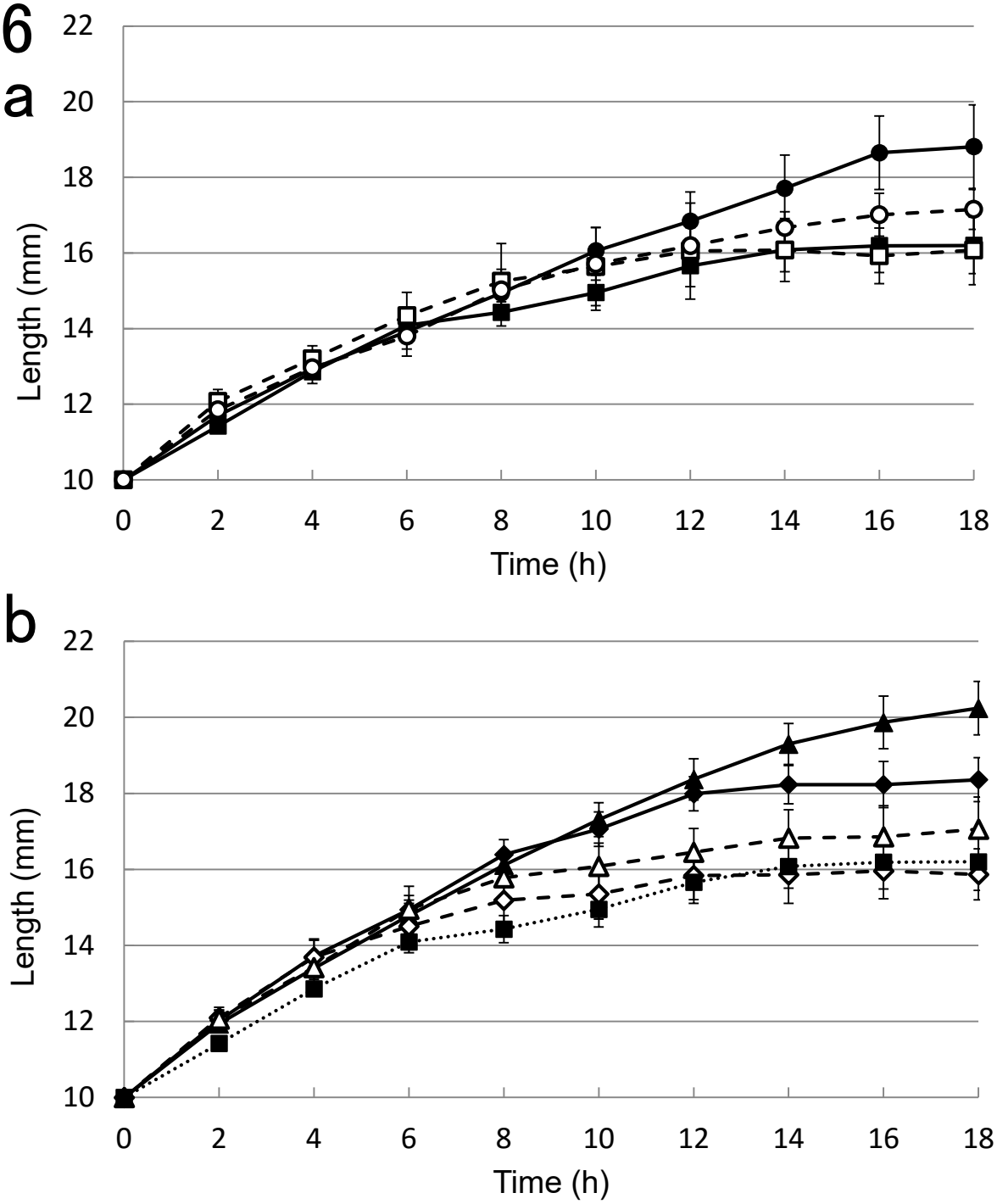

Fig. 6. Effect of colchicine on elongation induced by hormones and formic acid. Epicotyl segments were incubated in test solution with (open symbols) or without (closed symbols) 1×10^{-3} M colchicine in the presence of IAA (a) or IAA + GA_3 (b) (pH 4.0). Segment length was measured at the indicated times. Error bars indicate standard deviations (n = 10). Square, circle, diamond, and triangle indicate IAA, IAA + formic acid, IAA + GA_3, and IAA + GA_3 + formic acid, respectively.