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Cell Biology

Phytosulfokine, sulfated peptides that induce the proliferation of single mesophyll cells of *Asparagus officinalis* L.

(conditioned medium/growth factor/mesophyll cell/asparagus)

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ABSTRACT **Proliferation of dispersed plant cells in culture is strictly dependent on cell density, and cells in a low-density culture can only grow in the presence of conditioned medium (CM). No known plant hormones have been able to substitute for CM. To quantify the mitogenic activity of CM, we examined conditions for the assay system using mechanically dispersed mesophyll cells of *Asparagus officinalis* L. and established a highly sensitive bioassay method. By use of this method, the mitogenic activity of CM prepared from asparagus cells was characterized: it was heat-stable, susceptible to pronase digestion, and resistant to glycosidase treatment. On the basis of these results, the mitogenic activity in CM was purified 10^7-fold by column chromatography, and two factors named phytosulfokine-α and -β (PSK-α and PSK-β) were obtained. By amino acid sequence analysis and mass spectrometry, the structures of these two factors were determined to be sulfated pentapeptide (H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-OH) and sulfated tetrapeptide (H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-OH). PSK-α and PSK-β were prepared by chemical synthesis and enzymatic sulfation. The synthetic peptides exhibited the same activity as the natural factors, confirming the structure for PSK-α and PSK-β mentioned above. This is the first elucidation of the structure of a conditioned medium factor required for the growth of low-density plant cell cultures.**

The proliferation of plant cells in dispersed culture is strictly dependent on the initial cell density, and mitotic activity in low-density suspension cell cultures cannot be stimulated by supplementation with known plant hormones or defined nutrients. However, proliferation of plant cells at low density is induced by the addition of conditioned medium (CM) prepared from rapidly growing cells in culture (1). A possible explanation for this phenomenon is that a mitogenic factor or factors produced by the individual cells is essential for cell proliferation.

Since the late 1980s, detection and characterization of mitogenic factors that are often called conditioned medium factors (CMFs) have been performed in a few culture systems: highly hydrophilic, neutral compounds in CM of maize cell line (2, 3); highly hydrophilic, relatively heat-stable compounds in CM of a carrot cell line (4); and pronase-resistant, relatively small compounds in CM of *Pinus radiata* cells (5) have been reported. However, no factors have been isolated or identified in the above studies. The main obstacle in the purification and isolation of CMFs appears to be the method for monitoring activity. Available assay methods have not been sufficiently rapid or sensitive.

To solve this problem, we have used a primary culture system of mesophyll cells prepared from *Asparagus officinalis* L. Mechanically dispersed asparagus cells have relatively high viability and can vigorously proliferate in liquid medium (6, 7). We examined this cell system and established a highly sensitive bioassay system to detect the activity of CMFs. By use of this bioassay method, we characterized the chemical properties of CMFs of asparagus and purified two active compounds by three steps of chromatography. Chemical structures of these compounds were determined to be sulfated peptides and were confirmed by chemical synthesis. Our results indicate that peptidal growth factors, which are prominent in animal cell cultures, also exist in the plant kingdom.

MATERIALS AND METHODS

Plant. Seeds of *A. officinalis* L. cv. Mary Washington 500W (Takii Shubyo, Japan) were planted on moist sterile soil and kept in a growth room at 25 ± 2°C under a daily 16-h light period [Toshiba (Tokyo) DR 400/T and Mitsubishi (Yokohama) MLRBOC 400 F-U lamps; ≈20,000 lux at plant level]. The cladodes of 40- to 60-day-old spears were used as the experimental material. Cell lines of *Zea mays* L. and *Oriza sativa* L. were maintained with weekly subculturing in Murashige and Skoog medium supplemented with 1.0 mg of 2,4-dichlorophenoxyacetic acid per liter and 30 g of sucrose per liter. Cultures were incubated at 25°C in the dark, with rotary shaking at 120 rpm.

Bioassay. Approximately 0.5 g of cladodes were sterilized by soaking them first in 70% ethanol for 30 s, then soaking them in a solution of NaOCl (0.25%) for 10 min, and finally rinsing them three times with sterile water. Single cells were liberated from these cladodes by homogenization in a glass homogenizer containing sterile water. The homogenate was filtered through a 37-μm stainless mesh, and the filtrate was centrifuged at 100 × *g* for 3 min. The precipitated single cells were washed with sterile water three times and used in the experiments. From 0.5 g of fresh cladodes, ≈10^7 cells were obtained.

The basal medium used for cell culture, which contains 1.0 g of glutamine per liter, 10 g of sucrose per liter, 30 g of mannitol per liter, 1.0 mg of 1-naphthaleneacetic acid per liter, 0.3 mg of 6-benzyladenine per liter, inorganic salts, and trace elements, was prepared according to Paul *et al.* (7). Dispersed single mesophyll cells were suspended in sterilized water, adjusted to twice the final cell density with the hemacytometer, and dispensed into 24-well microplates at a volume of 250 μl per well. Culture medium (125 μl) prepared at 4-fold concentration, and various sample solutions (125 μl) were sterilized by filtration then gently added to the cell suspension in each well. Unless otherwise stated, specified bioassays were performed at an initial cell density of 4.0×10^4 cells/ml. The plates were sealed with Parafilm to avoid evaporation of the

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Abbreviations: CM, conditioned medium; CMF, conditioned medium factor; PSK, phytosulfokine; FAB-MS, fast atom bombardment mass spectrum or spectrometry.

Data deposition: The sequence reported in this paper has been deposited in the Protein Identification Resource data base (accession no. JT0870).

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medium and then incubated in the dark at 25°C with continuous rotary shaking at 120 rpm. Mitotic or mitogenic activity and cell viability were determined on the 6th day of culture by counting the number of nondivided cells, dead cells, and colonies (division into two or more cells) under an inverted microscope. Cell viability was calculated by dividing the number of live cells (nondivided cells and colonies) by the total number of cells observed (live and dead cells). Mitotic or mitogenic activity for each well was calculated by dividing the number of colonies by the number of live cells. ED_{50} value was defined as the concentration of the active compound required for 50% mitogenic activity on the 6th day of culture. All the treatments were represented in triplicate and errors calculated as standard deviations.

Preparation of CM. Single mesophyll cells were suspended in 200 ml of culture medium at a density of 2.5×10^5 cells/ml. This suspension was cultured in 500-ml Erlenmeyer flasks in the dark at 25°C with rotary shaking at 120 rpm. For a measurement of the cell growth curve, 10 ml of the cell suspension was collected every other day from the suspension cultures of dispersed mesophyll cells. The cells and CM were separated by centrifugation at $100 \times g$ for 10 min. The packed cells were dried in an evaporator, after which their weight was measured. For the purification of CMFs, 10-day CM was prepared and stored at −30°C.

Dialysis and Enzymatic Digestion. Five milliliters of CM was dialyzed with a dialysis membrane (Spectra/Por MWCO 1000; Spectrum Laboratories, Houston) against 500 ml of distilled water at 4°C for 12 h. The dialysate was concentrated to 10 ml, and the retentate was diluted to 10 ml, after which these two fractions were bioassayed.

Pronase E (Sigma) was dissolved in 20 mM KH_2PO_4-KOH buffer pH 7.5 at a concentration of 1.0 mg/ml. One milliliter of CM was lyophilized and then dissolved in 1.9 ml of the buffer, after which it was mixed with 0.1 ml of the enzyme solution, inactivated enzyme solution, or buffer and incubated at 37°C for 3 h. Enzymes were inactivated at 100°C for 10 min after digestion. These samples then were bioassayed. Similarly, Glycosidases "mixed" from *Turbo cornutus* (Seikagaku Kogyo, Tokyo) were dissolved in 20 mM glutamic acid-KOH buffer pH 4.0 at the concentration of 1.0 mg/ml. The digestion experiments were done by the method used for pronase E.

Purification of the Factors. Six hundred milliliters of CM was concentrated to 300 ml, adjusted to pH 7.4 with 6.0 N KOH, and then applied to a DEAE Sephadex A-25 column (2.5 × 19 cm, Pharmacia), which was first equilibrated with 20 mM Tris·HCl buffer at pH 7.4. The column was washed with 200 ml of equilibration buffer, and fractions were eluted successively with 200 ml of the buffer containing 250, 500, 750, 1000, or 1250 mM KCl. Each fraction was desalted by dialysis (Spectra/Por MWCO, 1000). Desalted active fractions recovered from the DEAE Sephadex column (1000 and 1250 mM KCl fractions) were lyophilized, dissolved in 1.0 ml of 20 mM KH_2PO_4-KOH (pH 5.8), and then applied to a Bio-Gel P-2 extra fine column (1.7 × 37 cm, Bio-Rad, Hercules, CA), which had been equilibrated with the same buffer. Fractions were eluted with the buffer at a flow rate of 15 ml/h with absorbance monitored at UV 220 nm. Five-milliliter fractions were collected, and each was bioassayed. The active fraction recovered from the Bio-Gel column was lyophilized, dissolved in 200 μl of 10% acetonitrile containing 0.1% trifluoroacetic acid, and chromatographed on a Develosil ODS-HG-5 column (4.6 × 250 mm; Nomura Chemical, Seto, Japan) by an isocratic elution of 10% acetonitrile containing 0.1% trifluoroacetic acid at a flow rate of 1.0 ml/min with monitoring the absorbance at 220 nm. Two-milliliter fractions were collected and lyophilized, and each was bioassayed. The two active peaks eluted at 9.6 and 14.5 min were collected and analyzed for amino acid sequences.

Amino Acid Sequence Analysis and Mass Analysis. Amino acid sequence was determined by Edman degradation with a gas-phase sequencer (Applied Biosystems; model 476A). Phenylthiohydantoin derivatives of amino acids obtained at each cycle of the Edman degradation were determined by reverse-phase HPLC on Applied Biosystems Brownlee C-18. Fast atom bombardment mass spectra (FAB-MS) were obtained by adding 1 μg of peptide in distilled water (1.0 μl) to glycerol (1.0 μl) on a stainless prove, followed by bombardment with 6 kv Xe fast atom on a mass spectrometer (JEOL; model DX-705L).

Peptide Synthesis. Unsulfated peptide was synthesized with the use of FastMoc chemistry with a peptide synthesizer (Applied Biosystems; model 433A) on 4-hydroxymethylphenoxymethyl resin according to the manufacturer's protocol. The sulfation of synthesized peptide was conducted with *p*-nitrophenyl sulfate used as the donor substrate. The reaction mixture contained 0.1 M glycine-NaOH (pH 8.6), 1 mM *p*-nitrophenyl sulfate, 0.2 mM synthesized peptide, 1.0 units/ml arylsulfotransferase, and 25 mM $MgCl_2$ in a total volume of 10 ml. The reaction was started by the addition of *p*-nitrophenyl sulfate, and the incubation was carried out at 37°C for 24 h. Sulfated peptide was purified on a Develosil ODS-HG-5 column (4.6 × 250 mm) by an isocratic elution with 10% acetonitrile containing 0.1% trifluoroacetic acid at a flow rate of 1.0 ml/min.

RESULTS

Establishment of the Bioassay System. The mitotic activity of single cells in suspension culture was markedly affected by the initial cell density (Fig. 1). At the initial cell density range of 1.3–5.0 × 10^5 cells/ml, cell division was observed from the 3rd day of culture, and all the cells formed the colonies until the 10th day of culture. At lower densities, the mitotic activity decreased sharply with the decrease in the initial cell density. Approximately 70% of the cells were alive after 6 days of culture regardless of the initial cell densities. These results indicate that the proliferation of mechanically dispersed single mesophyll cells of asparagus depends strictly on the initial cell density and that the factor or factors produced by individual asparagus cells are indispensable for cell proliferation.

To detect the mitogenic activity of the factor or factors, we cultured single cells at the suboptimal density of 4.0×10^4 cells/ml in the presence of various concentrations of CM prepared from a 10-day suspension culture of asparagus cells. The ratio of divided cells was markedly increased by small amount of additions of CM. The mitogenic activity was ≈70% at a final CM concentration of 12.5% (vol/vol) added on the 10th day after the start of the bioassay (Figs. 2 and 3). Only 5–7

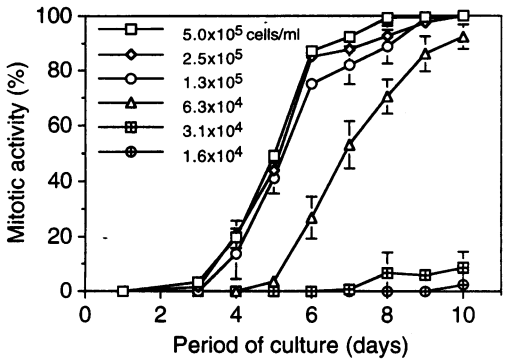

FIG. 1. Effect of the initial cell density on cell proliferation. Single cells were suspended in liquid medium, dispensed into 24-well culture plates at a final volume of 0.5 ml per well, and incubated at 25°C with shaking at 120 rpm. The mitotic activity for each well was calculated by dividing the total number of colonies (division into two or more cells) by the total number of living cells.

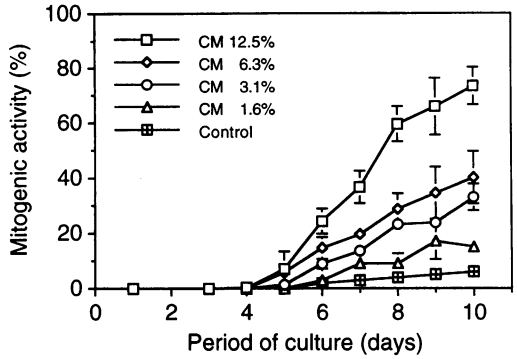

FIG. 2. Effect of CM on cell proliferation. Single cells were incubated in the liquid medium at a density of 4.0×10^4 cells/ml in the presence of various concentrations of CM.

days are required to determine the mitogenic activity of the factor or factors with this system, and the minimum detectable concentration of CM is ≈1.6%. The sensitivity of dispersed mesophyll cells to CMFs is exceptionally high as compared with those obtained in a bioassay using protoplasts or cells derived from callus (2–4). This system is extremely useful for the detection of the activity of the factor or factors.

By use of this bioassay, we measured the accumulation of the factors in CM from the 2nd to 18th day of culture and correlated it with the growth kinetics of suspension cells.

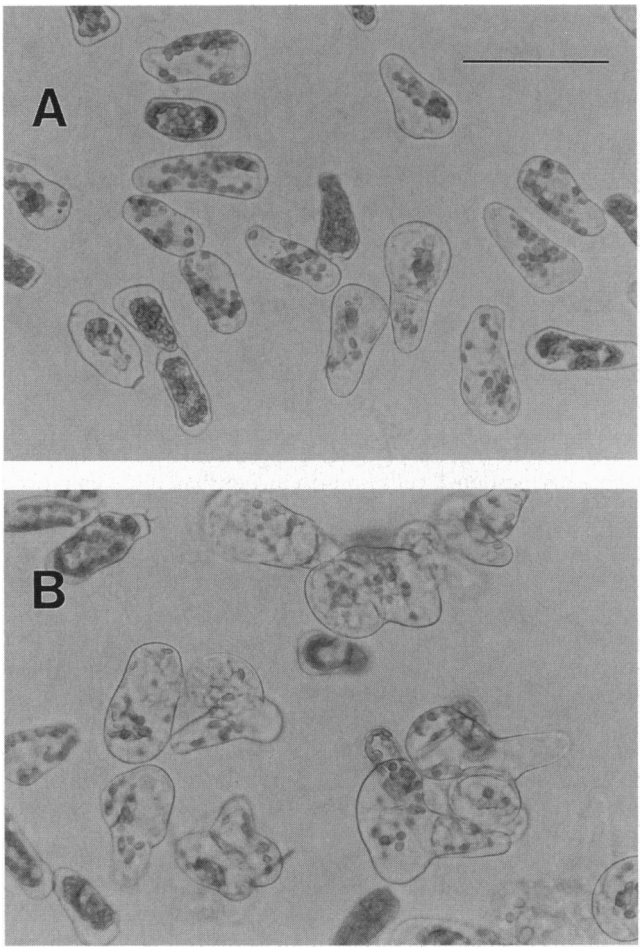

FIG. 3. Micrographs of the asparagus mesophyll cells. (*A*) Cells cultured without CM, showing only undivided cells. (*B*) Cells cultured with 12.5% of CM, showing many divided cells. The initial cell density was 4.0×10^4 cells/ml. Cells were observed after 8 days of culture. Scale bar = 50 μm.

Dispersed mesophyll cells had an exponential growth stage with a doubling time of 72 h from the 6th to 12th day of culture. Dry cell weight on the 18th day was ≈10-fold the initial weight. The mitogenic activity of the factor or factors in CM increased with the growth of the cultured mesophyll cells, reaching its maximum on the 10th day of culture (Fig. 4). We therefore used 10-day CM in the following experiments to characterize and purify the factors. The decrease of the CM activity after the 12th day of culture can be explained by degradation of the active compounds or the accumulation of compounds inhibitory to the cell proliferation.

Characterization of the Factor or Factors. To characterize the chemical properties of the factor or factors, we subjected CM to stability tests, dialysis, and enzymatic digestion. About 90% of the activity of the factor or factors was retained after incubation at 100°C for 10 min. When CM was dialyzed against a membrane (Spectra/Por MWCO, 1000), all the activity was retained inside of the membrane, suggesting that M_r of the factor or factors would be more than 1000 if it were not for ionic repulsion between the factor and the dialysis membranes weak negative charge. After incubation of CM with pronase E, the activity of the factor or factors was completely lost, indicating that a peptide moiety in its molecule is important for mitogenic activity. On the other hand, the activity was fully retained after the incubation of CM with a glycosidase mixture, suggesting that the factor or factors probably do not have an oligosaccharide moiety.

Purification of the Factors. Based on the results of the characterization studies, we established the purification procedure of the factor or factors from CM by two steps of open column and one step of the HPLC system. CM was first fractionated by stepwise elution from a DEAE-Sephadex column. The active factor or factors were strongly adsorbed on the column and were found in the 1000 mM and 1250 mM KCl fractions. These fractions were desalted by dialysis and purified on a gel permeation column of Bio-Gel P-2. The factor or factors were eluted in the 40- to 45-ml fraction (Fig. 5*A*). The apparent M_r of this factor or factors was estimated as ≈1300 by comparison with size markers. Active fractions from the gel permeation column were further purified on a reverse-phase HPLC column, and two active compounds eluted at 9.6 and 14.5 min were obtained (Fig. 5*B*). We named these compounds phytosulfokine-α (PSK-α) and PSK-β. The total yields of PSK-α and PSK-β were 2 μg and 10 μg, respectively, from 600 ml of CM. A purification of $\approx 10^7$-fold has been achieved, with a recovery of activity of about 10%. The minimum concentration of PSK-α exhibiting the mitogenic activity was 1.0×10^{-9} M, and ED_{50} was 3.8×10^{-9} M. PSK-β exhibited its

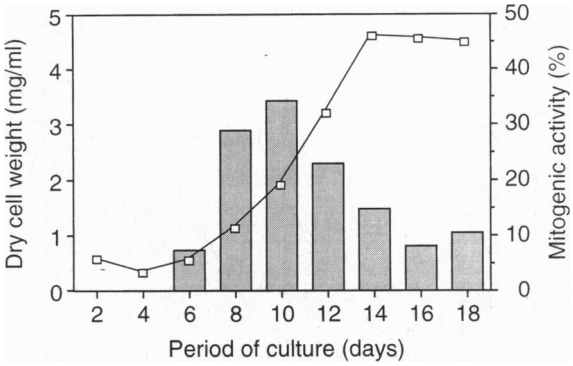

FIG. 4. Cell growth curve and mitogenic activity of CM on each culture stage. Single cells were suspended in culture medium at a density of 2.5×10^5 cells/ml and were cultured at 25°C with shaking at 120 rpm. Ten milliliters of the cell suspension was collected every other day from the suspension cultures. Mitogenic activity of CM obtained at each growth stage (right scale, shaded bars) was correlated with the growth kinetics of suspension cells (left scale, open squares).

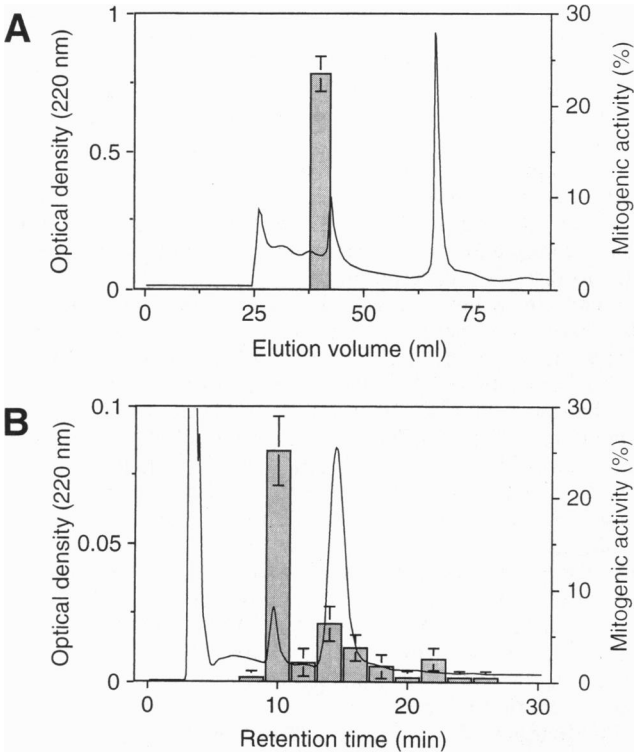

FIG. 5. Purification procedure of PSK-α and PSK-β. (*A*) Desalted active fractions recovered from the DEAE-Sephadex column were lyophilized and chromatographed on a Bio-Gel P-2 extra-fine column. Fractions were eluted with the buffer, and absorbance was monitored at UV 220 nm (left scale, solid line). Mitogenic activity (right scale, shaded bars) was determined after 6 days of culture. (*B*) Active fraction recovered from the Bio-Gel column was chromatographed on a Develosil ODS-HG-5 column. The two active peaks eluted at 9.6 and 14.5 min (PSK-α and PSK-β) were collected and analyzed for amino acid sequences.

activity at a concentration of 1.0×10^{-8} M, and ED_{50} was 3.7×10^{-8} M (Fig. 6).

Structure Determination of PSK-α and PSK-β. Amino acid sequence analysis of PSK-α with a gas-phase amino acid sequencer yielded the primary structure Tyr-Ile-Tyr-Thr-Gln. FAB-MS analysis showed, however, that the M_r of PSK-α was 846, which was 160 mass units higher than the M_r expected from the established amino acid sequence. In addition, a pseudomolecular ion corresponding to $[M-2H+K]^-$ was observed at m/z 883, and a major fragment ion corresponding to $[M-H-80]^-$ was observed at m/z 765. These results indicated

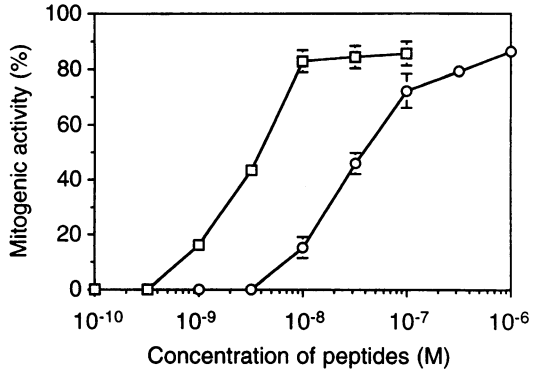

FIG. 6. Mitogenic activities of the natural PSK-α (open squares) and PSK-β (open circles). Single cells of asparagus were cultured at a cell density of 4.0×10^4 cells/ml in the liquid medium containing various concentrations of natural PSK-α and PSK-β.

that PSK-α is a chemically modified peptide and that modification can be easily removed under the condition of the amino acid sequence analysis.

Purification experiments described above suggested that PSK-α has a strongly acidic group or groups in the molecule. Observation of the fragment ion $[M-H-80]^-$ in the negative FAB-MS experiments indicated that PSK-α is a sulfated compound (8), and the additional 160 mass units of PSK-α would be explained if both tyrosine residues were *O*-sulfated. Sulfation of the tyrosine residues is often observed in extracellular-secreted peptides (9, 10), and sulfated peptides are reported to be strongly adsorbed on a DEAE-Sephadex column, as is PSK-α (11, 12). The sulfated group of *O*-sulfated tyrosine is reported to be readily removed under acidic conditions, such as those used in sequence analysis (13). Thus, we surmised that the structure of PSK-α is H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-OH.

We synthesized a peptide with the proposed structure of PSK-α to confirm this chemical structure. The unsulfated peptide was synthesized by a peptide synthesizer, and sulfate groups were incorporated on the peptide by arylsulfotransferase (14). This enzyme catalyzes the transfer of a sulfate group from phenolic sulfate ester to other phenolic compounds with strict specificity. The synthetic disulfated peptide was eluted at the same retention time as that of natural PSK-α on reverse-phase HPLC, and its mass spectra were identical to those of natural PSK-α in negative FAB-MS. This peptide was as effective as the natural PSK-α for inducing cell proliferation. The terminal amide analog of PSK-α prepared by the above procedure for PSK-α was eluted at a different retention time from reverse-phase HPLC and showed lower activity than that of PSK-α. In addition, the unsulfated analog of PSK-α was inactive up to 1.0×10^{-7} M. The complete structure of PSK-α was, therefore, established as H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-OH. Similarly, the structure of PSK-β was determined to be H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-OH by the same method employed for PSK-α. PSK-β was, thus, likely an enzymatic degradation product of PSK-α.

DISCUSSION

In previous studies on CMFs, callus-derived suspension cells or protoplasts have been widely used as the plant material in bioassay systems that were relatively insensitive and time-consuming (1–5). The sensitivity to mitogenic activities obtained by our bioassay system using mechanically dispersed asparagus mesophyll cells is much higher than those employed in previous reports. Since the initial cell population is composed of only single mesophyll cells in this bioassay system, it is easy to observe with a microscope whether each cell has divided or not. Our system, therefore, is extremely useful for detecting mitogenic activity of the CMFs produced by asparagus cells. Using this method, we demonstrated that the proliferation of the asparagus cells is strictly dependent on the extracellular concentration of PSKs. Even when the initial cell population is lower than the critical cell density, rapid cell proliferation is observed by incubating the cells in the presence of PSKs and continues until many cells form visible colonies. PSKs could be useful for plant regeneration from transgenic or fused cells.

There have been some arguments as to whether CMFs correspond to a single compound or the mixture of a few compounds (15, 16). It has also been reported that some medium supplements, such as amino acids, ammonium ion, and purine derivatives promote cell proliferation (17–19). We, however, observed no dispersal over several fractions or serious decrease in the mitogenic activity of CMFs in the purification experiments that would be expected if the CMF was composed of several components acting synergistically.

The PSKs described above are most probably the main active principles of CMFs of asparagus.

All the cells employed in our experimental system are derived from asparagus mesophyll cells and are considered to be homogeneous, as shown in Fig. 3. Consequently, PSKs would be an autocrine-type cell growth factor that is often observed in mammalian cells (20). Two of the most attractive physiological issues are whether PSKs are produced only in cell culture or play a vital role in the intact asparagus plant and what stimulus triggers the production of PSKs. In many plant species, mechanical wounding activates a set of genes related to defense mechanisms, such as a key enzyme of ethylene synthesis (21), cell wall proteins (22), and protease inhibitors (23, 24). In 1991, an oligopeptide systemin that induces the synthesis of two proteinase inhibitor proteins in tomato plants was isolated from wounded tomato leaves (25). This peptide was transmitted from the wounded to the unwounded leaves as quickly as 20 min after wounding. Our experimental system uses asparagus mesophyll cells, which are mechanically dispersed just before use. Each single mesophyll cell may receive the mechanical wounding, but we do not know as yet whether the production of PSKs are related to wounding. One significant observation is that the mitogenic activities of PSKs in CM are not detected until the 6th day of culture and reach their maximum in the middle of the exponential phase of the cell growth curve (Fig. 4). Similar results were reported in the suspension culture of cell line of *Z. mays* L. (3). PSKs, therefore, may not be produced as the primary response to mechanical wounding. The relationship between the production of PSKs and the wound response of plant cells requires further investigation.

Previous reports have shown that cell proliferation is promoted not only by CMFs produced by homospecific cells but also by those produced by some heterospecific cells (3, 16, 26, 27). These crossfeeding effects, however, are not generally observed between monocots and dicots, suggesting that CMFs are specific for monocots and for dicots. Our preliminary studies show that partially purified CM prepared from the suspension culture of *Z. mays* L. and *O. sativa* L. also induce the proliferation of asparagus mesophyll cells. The active fractions bioassayed by the asparagus system elute from DEAE-Sephadex at the same KCl concentrations as PSKs. The relative activities of these fractions are 13% (*Z. mays* L.) and 102% (*O. sativa* L.), compared with the mitogenic activity of asparagus CM. PSKs or PSK-like compounds may exist in all monocots.

Various tyrosine sulfate containing peptides have been isolated from vertebrate and invertebrate tissues, and the sulfation of tyrosine residue in peptides has been recognized as one of the general posttranslational modifications (28). It has been reported that two sulfated peptides, CCK (29) and leucosulfakinin (13), require the sulfate ester on tyrosine residues for the expression of their biological activities, although the essential function of the sulfate ester group is still unclear. In this paper, we also demonstrated that sulfated peptides have a vital role in plant cells and that the sulfate groups of PSKs are indispensable for their activity. The presence of two tyrosine *O*-sulfates is characteristic of PSKs, and such a disulfated peptide is novel. No significant similarities have been found to known protein sequences in homology searches of GenBank using the BLAST program. Biosynthetic studies of PSKs may provide novel information about the consensus sequence for sulfation and mechanisms of membrane transport in higher plants. PSK-β, which is the C-terminal truncated tetrapeptide of PSK-α, shows relatively high activity, indicating that the C-terminal amino acid of PSK-α is not essential for the expression of its activity. Structure-function studies are now in progress.

In mammalian cells, many peptidal growth factors have been identified, and the investigations of their physiological roles have led to the clarification of the mechanisms of cell growth and differentiation. However, no endogenous peptidal factor that qualifies as a "growth factor" has been isolated from a higher plant. PSKs are the first chemically characterized growth factors of higher plant, and further analysis of their physiological functions should provide new avenues for investigating the mechanisms controlling cell proliferation in higher plants.

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