magma complex. At a depth of 5 km (Fig. 2C), the high-velocity core of Kilauea is clearly evident, extending along the upper east and southwest rifts and intruding into the Koae area. Kaoiki and other nonrift areas continue to display rather low velocities. By 8 km the model resolution has become somewhat poor, but the high velocities of the volcano core are still evident, apparently with a slight southward shift from the shallower levels. The very high velocity (> 8 km/sec) directly beneath the caldera is consistent with the results of a previous refraction study (*3*).

An east-west cross section through the caldera (at y = 16 km; Fig. 3) shows the association between the velocity structure and seismicity in the caldera area. Earthquakes cluster around the low-velocity zone directly beneath the caldera at shallow depth. The aseismic zone coincides precisely with the zone of low P-wave velocity. Thus the summit magma complex of Kilauea is detectable in the seismic velocity structure. The low velocity is most likely due to a combination of elevated temperature and the presence of partial melt and pockets of magma.

To summarize, starting with the caldera area, we find near-surface high velocities underlain by a zone of relatively low velocity that displays a slight southward shift with increasing depth. We interpret this as the roof and main body of Kilauea's summit magma complex. The low-velocity zone coincides with an aseismic zone beneath the caldera. The surrounding volume is characterized by rather high velocities, corresponding to the core of the volcano. Systematically high velocities are also observed along the upper east and southwest rift zones. High velocities along the Koae fault system are clear evidence for intrusive activity there. The nonrift areas, especially around the Kaoiki fault system, display almost uniformly low velocities. These findings are generally consistent with previous refraction (*3, 4*) and teleseismic (*5*) studies, but indicate the power of the simultaneous inversion method to reveal three-dimensional structure on a finer scale than can be obtained otherwise.

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17 October 1983; accepted 14 November 1983

Calcium-Promoted Protein Phosphorylation in Plants

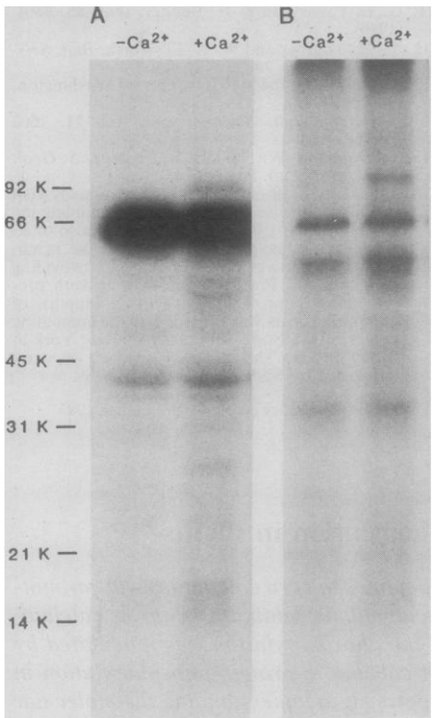
Abstract. *Phosphorylation of several polypeptides in corn coleoptiles was promoted by adding calcium. Chlorpromazine, a calmodulin inhibitor, reduced calcium-promoted phosphorylation, suggesting that the phosphorylation was modulated by calmodulin. This is evidence for the role of calcium in protein phosphorylation in plants and could serve as an experimental approach to understanding the molecular mechanism by which calcium modulates various physiological processes in plants.*

In order to adapt efficiently to the changing environment, all living organisms have built-in regulatory mechanisms that can be controlled in response to external stimuli. Extensive information from animal systems indicates that hormonal and neural stimuli are the two most important means by which individual cells respond to messages from the rest of the organism. The message received on the surface of individual cells is transmitted to the metabolic machinery of the cell by second messengers, such as adenosine 3′,5′-monophosphate (cyclic AMP) and calcium. Attempts have been made to explore the role of cyclic AMP as a second messenger in plants. Although convincing evidence was obtained for the existence of cyclic AMP in plants, sufficient evidence could not be obtained for its physiological role as a second messenger (*1*). Therefore, there is increasing interest in defining the possible role of calcium as a second messenger in plants (*2*).

Calcium affects several physiological processes in plants. For example, it has been implicated in geotropism (*3*), cell elongation, cell division, protoplasmic streaming, senescence, abscission, α-amylase secretion, and several other processes (*4*). In animals, calcium has been shown to regulate various biochemical processes after binding to proteins like calmodulin (*5*).

Occurrence of calmodulin in plants has been well documented, and calcium- and calmodulin-modulated activation of enzymes such as nicotinamide adenine dinucleotide (NAD^+) kinase, Ca^{2+}-activated adenosinetriphosphatase (ATPase), and isofloridoside phosphate synthase has been reported from plant systems (*6*). Studies with calmodulin inhibitors suggest the possible involvement of calcium and calmodulin in various hormonal responses and geotropism (*7*). Recently, evidence has been obtained for the presence of endogenous calmodulin inhibitors in plants that could regulate calmodulin activity in vivo (*7*). Protein phosphorylation is now emerging as the major mechanism by which calcium and calmodulin regulate biochemical events inside the cell in response to external stimuli (*8*). Many protein kinases that are activated by calmodulin in the presence of calcium have been reported from animal cells. However, only limited information is available about the role of calcium and calmodulin in the phosphorylation of proteins from plant systems (*9*). We now report evidence for the role of calcium in the phosphorylation of soluble and membrane proteins of corn coleoptiles. Phosphorylation of thylakoid proteins, nuclear proteins, wheat germ proteins, and cytokinin receptors from plants have been reported (*9*); however, we know of no evidence for the role of calcium in the phosphorylation of those proteins.

Soluble and membrane proteins were isolated from corn coleoptiles in a buffer containing 0.2 m*M* EGTA (*10*). Phosphorylation of proteins was performed in the presence of [γ-^{32}P]ATP. Phosphorylated proteins were analyzed by poly-



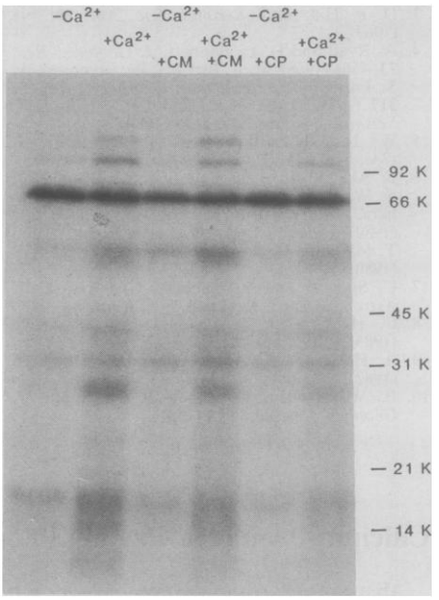


Fig. 1 (left). Effect of Ca^{2+} on the phosphorylation of soluble proteins (A) and membrane proteins (B) from corn coleoptiles. Corn (*Zea mays* var. patriot) seeds were germinated on vermiculite in the dark. Coleoptiles were harvested after 6 days and homogenized with mortar and pestle with an equal volume of medium 1 containing 50 m*M* 2-(*N*-morpholino)ethane sulfonic acid (MES) and NaOH (*p*H 7.0), 5 m*M* $MgCl_2$, 0.5 m*M* dithiothreitol, 0.25*M* sucrose, and 0.2 m*M* EGTA. All operations were performed at 4°C. After centrifugation for 10 minutes at 7000*g* the supernatant was again centrifuged at 130,000*g*. The pellet (the membrane fraction) was then suspended in medium 2 (50 m*M* MES-NaOH, 5 m*M* $MgCl_2$, 0.1 m*M* dithiothreitol, and 0.2 m*M* EGTA) and centrifuged at 130,000*g*. Washing was repeated and the final pellet was suspended in medium 2 (1 ml per 5 g of initial fresh weight) and homogenized in a glass homogenizer. The membrane fraction contained 4.4 mg of protein per milliliter. The soluble protein fraction from the supernatant obtained by centrifugation at 130,000*g* was dialyzed against medium 2, centrifuged at 27,000*g*, and concentrated five times in an Amicon Stirred Cell with a PM-10 filter. The final preparation of soluble protein contained 6.7 mg of protein per milliliter. The reaction mixture for assaying protein phosphorylation (final volume, 130 μl) contained 0.44 mg of membrane protein or 0.67 mg of soluble protein, 50 m*M* MES-NaOH, 5 m*M* $MgCl_2$, 0.1 m*M* dithiothreitol, 0.2 m*M* EGTA (minus Ca^{2+}) or 0.2 m*M* EGTA and 0.5 m*M* $CaCl_2$ (plus Ca^{2+}), and 0.25 nmole of [γ-^{32}P]ATP (30 to 40 mCi/mmole). Prior to the addition of [γ-^{32}P]ATP, the reaction mixture was preincubated for 1 minute at 30°C, and phosphorylation was initiated by adding [γ-^{32}P]ATP. Incubation was carried out for 1 minute at 30°C; the reaction was terminated by adding an equal volume of electrophoresis sample buffer (*11*) and heating the preparation for 5 minutes in a boiling water bath. A 40-μl portion was analyzed by electrophoresis on 8 to 16 percent linear gradient gels (*11*). Kodak X-Omat AR film was exposed to the dried gels for 3 to 4 days for autoradiography. Fig. 2 (right). Effect of chlorpromazine on Ca^{2+}-stimulated phosphorylation of soluble proteins. Soluble proteins were extracted from corn coleoptiles stored at −20°C. Preparation of soluble proteins, phosphorylation, and analysis by gel electrophoresis were performed as indicated in the legend to Fig. 1. Bovine brain calmodulin (*CM*) (20 μg/ml) and chlorpromazine (*CP*) (0.2 m*M*) were added when required.

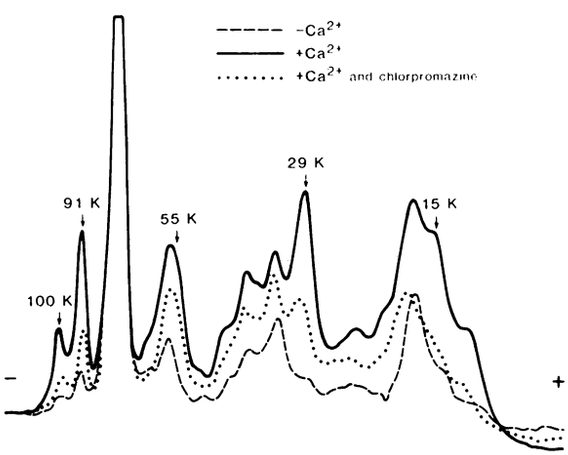


Fig. 3. Results of densitometer scanning (Quick Quant II) of lanes 1, 2, and 6 in Fig. 2. Molecular weights of the phosphorylated proteins are indicated.

acrylamide gel electrophoresis in buffers containing sodium dodecyl sulfate (*11*). Labeled proteins were detected by autoradiography.

Phosphorylation of a number of soluble polypeptides was markedly increased in the presence of calcium (Fig. 1A). Promotion of phosphorylation was evident in polypeptides with molecular weights of 91,000, 55,000, 29,000, and 15,000. Phosphorylation of a few proteins was not altered in the presence of calcium. Heating the extract for 5 minutes in a boiling water bath before the phosphorylation assay prevented phosphorylation of any proteins. Phosphorylation of membrane polypeptides with molecular weights of 85,000 and 16,000 was markedly promoted in the presence of calcium (Fig. 1B). Heating the membranes for 5 minutes in a boiling water bath before phosphorylation abolished all phosphorylation (*12*).

The role of calmodulin in calcium-modulated protein phosphorylation was investigated by using the calmodulin inhibitor chlorpromazine (*13*). Calcium markedly promoted the phosphorylation of polypeptides with molecular weights of 100,000, 91,000, 55,000, 29,000, and 15,000 (Fig. 2). Addition of calmodulin did not alter the pattern of protein phosphorylation, suggesting that the endogenous calmodulin level is sufficient to modulate calcium-promoted phosphorylation. The presence of chlorpromazine did not affect calcium-independent phosphorylation. However, calcium-promoted phosphorylation was markedly reduced in the presence of chlorpromazine. Densitometer scanning of the autoradiograph in Fig. 2 is shown in Fig. 3. Calcium-stimulated phosphorylation of all polypeptides was reduced. Particularly, the 29,000-dalton polypeptide that showed marked promotion of phosphorylation in the presence of calcium exhibited pronounced inhibition in the presence of chlorpromazine.

To confirm that phosphorylation occurred on proteins and not on other macromolecules such as nucleic acids, the phosphorylated polypeptides in electrophoresis sample buffer were treated with Pronase (200 μg/ml) for 6 hours at 30°C. After electrophoresis, protein staining did not show any protein bands, and autoradiography did not reveal any radioactive bands. Therefore, the radioactive bands were due to phosphorylation of proteins. The possibility that the phosphoproteins represent intermediate stages of enzyme catalysis, as occurs in Ca^{2+}-activated ATPase, is not ruled out (*14*), but is unlikely because of the calcium-promoted phosphorylation of several

polypeptides. Whether phosphorylation occurs on serine, threonine, or tyrosine residues is also not known.

Recent experimental evidence indicates that calcium- and calmodulin-modulated protein phosphorylation is the general mechanism mediating the neural response in mammalian tissues (*8*). Dephosphorylation of the phosphorylated proteins by specific phosphoprotein phosphatases offers a mechanism by which the biochemical pathways involving phosphorylation can be deactivated. Several investigators (*15*) have obtained evidence in plants for the regulation by phosphorylation and dephosphorylation of pyruvate dehydrogenase, hydroxymethylglutaryl–coenzyme A reductase, and quinate:NAD^+ 3-oxidoreductase (*15*); however, the role of calcium and calmodulin was not demonstrated. Lin *et al.* (*16*) reported phosphoprotein phosphatase from plants but did not indicate its link with calcium.

These findings on calcium-promoted protein phosphorylation should advance our understanding of the molecular mechanisms by which calcium regulates physiological processes such as phototropism, gravitropism, cytoplasmic streaming, chromosome movement, and other plant movements in response to various stimuli. Additional research is required to elucidate the nature of the protein kinases and substrates for protein kinases. Evidence for the existence of phosphoprotein phosphatase mediating selective dephosphorylation also needs to be obtained.

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13. Phosphorylation was studied in the soluble protein fraction from frozen coleoptiles in which calcium-independent protein phosphorylation was lower. Phosphorylation of the 100,000-dalton polypeptide was very low in extracts from fresh coleoptile but was pronounced in the extract from frozen coleoptiles.
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17 October 1983; accepted 4 November 1983

Nicotine Is Chemotactic for Neutrophils and Enhances Neutrophil Responsiveness to Chemotactic Peptides

Abstract. *Neutrophils contribute to chronic bronchitis and pulmonary emphysema associated with cigarette smoking. Nicotine was found to be chemotactic for human neutrophils but not monocytes, with a peak activity at ~ 31 micromolar. In lower concentrations (comparable to those in smokers' plasma), nicotine enhanced the response of neutrophils to two chemotactic peptides. In contrast to most other chemoattractants for neutrophils, however, nicotine did not affect degranulation or superoxide production. Nicotine thus may promote inflammation and consequent lung injury in smokers.*

Cigarette smoking is associated with the development of chronic bronchitis and pulmonary emphysema (*1*). Lung injury in smokers has been attributed to the effects of prolonged low-grade inflammation centered about the respiratory bronchioles and alveolar ducts (*2*) and especially to the release of polymorphonuclear neutrophil (PMN) elastase, an enzyme that produces emphysema when instilled intratracheally in animals (*3*). The recruitment of PMN's and mononuclear phagocytes to the airways of hamsters that have inhaled cigarette smoke (*4*) and the increased numbers of neutrophils in bronchoalveolar washings from human smokers (*5*) may be explained in part by the release of chemotactic factors from alveolar macrophages (*6*). However, a direct chemotactic effect of the components of cigarette smoke on inflammatory cells is an additional possibility. We now report that nicotine, which is readily absorbed from the respiratory tract and reaches plasma concentrations of 5 to 50 ng/ml (approximately 30 to 300 n*M*) in cigarette smokers (*7*), has effects on PMN's that may be relevant to lung inflammation in vivo.

We found that nicotine is chemotactic for human PMN's but not monocytes. Unlike many factors chemotactic for PMN's, it did not stimulate the release of superoxide anion (SOA) or cause degranulation. At low concentrations, nicotine enhanced directed migration of PMN's in response to optimum concentrations of complement-derived chemotactic activity (C5fr) and of *n*-formylmethionyl-leucyl phenylalanine (fMLP). As with its direct effect on PMN's, nicotine did not enhance fMLP-induced SOA release or degranulation.

Nicotine produced a concentration-dependent migratory response of PMN's obtained from heparinized venous blood of nonsmokers; the response was maximal at 30.8 μ*M* (Fig. 1A). The average net number of cells migrating in response to this optimum nicotine concentration was indistinguishable from the response to 10^{-8} *M* fMLP ($P > 0.3$, Student's *t*-test). When a concentration gradient for nicotine was not present (Fig. 1B), PMN migration was not different from that observed in chambers in which culture medium only was used in the lower compartment. These data indicate that nicotine is chemotactic for PMN's, producing a stimulus to directed migration comparable to that of the well-studied potent chemotactic factor fMLP. Under similar test conditions, preparations of human monocytes showed no chemotactic response to nicotine.

To determine whether nicotine might also modify the response of PMN's to other chemotactic factors, we incubated