## BRIEF PAPERS

# IDENTIFICATION OF PHOSPHORYL CHOLINE AS AN IMPORTANT CONSTITUENT OF PLANT SAPS[1,2]

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Several phosphorylated compounds have been reported in xylem saps of a variety of plants by Tolbert and Wiebe (8). They characterized the major compound by its $R_f$ value (0.9 in phenol:0.3 in butanol–propionic acid–water), apparent non-ionic character, acid stability and phosphatase hydrolysis to orthophosphate.

In studying phospholipid intermediates of plants we have found that phosphoryl choline is the major phosphate ester in plant saps. This compound has been identified in animal and bacterial phospholipid metabolism but has not been reported as a constituent of plant saps (1).

The major part of the labeled phosphoryl choline ($P^{32}C$) which occurs in barley leaves was extracted with 80 % ethanol and separated by two dimensional paper chromatography (4). The compound was detected by virtue of the radioactivity incorporated during growth of the seedling plants in $P^{32}$ nutrient or with $C^{14}$ incorporated by several days photosynthesis in $C^{14}O_2$. The very slow carbon labeling with $C^{14}O_2$ may be contrasted to the more rapid synthesis of $PC^{14}$ with labeled choline precursors. PC is identical with a labeled product of $HC^{14}OOH$ and $H_2C^{14}O$ feeding experiments. The unknown compound $U_1$ isolated from barley fed $HC^{14}OOH$ by Tolbert (9) is PC. Glycolic-2-$C^{14}$ is partially converted to PC when vacuum infiltrated into barley leaves.

Barley seedlings were placed in small vials of tap water containing 0.2 mc radiophosphate (pH 6). After 20 hours at 200 ft-c the leaves were cut and extracted with boiling 80 % ethanol. Plants to be labeled with $C^{14}$ and $P^{32}$ were placed in a test tube containing dilute $KH_2P^{32}O_4$ (0.2 mc) and $C^{14}O_2$ (0.1 mc). After five days (200 ft-c) the leaves were extracted. The concentrated extract from one gm of tissue was chromatographed on a sheet of Whatman No. 4 paper. A compound with $R_f$ corresponding to that reported by Tolbert and Wiebe was identified by cochromatography with authentic $P^{32}C$. The exudate from the cut stem was then chromatographed and found in the same manner to contain orthophosphate and $P^{32}C$. Authentic PC and glyceryl phosphoryl choline, GPC, were kindly supplied by Professor D. J. Hanahan of the University of Washington.

The $P^{32}C$ was acid-stable. Its hydrolysis rate, determined from measurements of the freed radiophosphate isolated chromatographically, was similar to that reported for PC by Baer and McArthur (2). "Polidase S" (Schwarz Chemical Company) hydrolysis gave orthophosphate and choline-$C^{14}$. The authentic carrier choline was detected by Draggendorf's reagent (5) ($KBiI_4$) which gave a reddish-brown spot coincident with the $C^{14}$ radioactivity.

The unknown compound was further characterized and identified as PC by electrochromatography with 0.1 M lactic acid (pH 2.5) as the electrolyte on filter paper strips (7). Neither PC nor the unknown compound migrated when on mixed or on separate origins, whereas orthophosphate migrated rapidly as an anion. When 0.1 M lactic acid adjusted to $pH < 1$ with hydrochloric acid was used as supporting electrolyte, PC and the unknown compound moved at slow, identical rates as cations while orthophosphoric acid migrated slowly as an anion. In 0.1 M potassium lactate adjusted to $pH > 11$ with KOH, PC and the unknown compound migrated slowly as an anion. Orthophosphate also migrated as an anion but much more rapidly than PC. All electrochromatographic studies (7) were run at a potential gradient of 7 volts per cm on strips of Whatman No. 4 filter paper 54 cm × 9 cm with origins at the middle. Nonradioactive compounds were detected using the spray reagent described previously (3) for detecting phosphorus. The $P^{32}C$ area was further resolved in methanol-ammonia-water solvent (60:10:30, v/v) (3) giving 10 to 15 % of a compound moving 18 % faster than PC and having the properties of glyceryl phosphoryl choline with which it chromatographed exactly.

The identification of phosphoryl choline in plant saps in amounts at least as high as 5 to 20 % (8) of the total phosphorus suggests its function as a phosphate carrier. The fact that it has now been found in phloem exudate after injection of radiophosphate into spinach and cucumber leaves indicates that the phosphorus transport by this means is not unidirectional. The phosphorylative mechanisms of plants occur in

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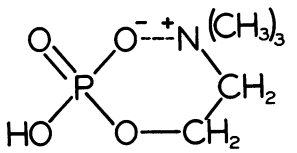

FIG. 1. Zwitter ion structure of phosphoryl choline.

phospholipid-containing structures, mitochondria and chloroplasts. The appearance of free PC and GPC suggests a relationship between phospholipid intermediates and possible mechanisms of phosphorylation. The transport of phosphorus as PC or GPC should be facilitated by the high organic-solubility, low adsorbtion and neutral character of these zwitter ions. Indeed, such a zwitter ion nature as indicated in figure 1 would lend itself to interpretation by the ion-carrier complex theory supported by Epstein (6). According to this theory ions combine with a carrier which enables them to traverse membranes of selective permeability after which they are released as free ions.

## SUMMARY

The major phosphate ester in plant saps has been identified chromatographically as phosphoryl choline. It was readily labeled with $P^{32}$ upon absorption of $KH_2P^{32}O_4$ through the roots and with $C^{14}$ by vacuum infiltration of formate-$C^{14}$, formaldehyde-$C^{14}$ and glycolate-2-$C^{14}$. However, $C^{14}$ was incorporated very slowly by $C^{14}O_2$ photosynthesis. The zwitter ion structure and organic solubility of phosphoryl choline suggests that it may act as a phosphorus carrier capable of penetrating plant membranes.

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# SOME PHENOTYPIC EFFECTS OF SINGLE GENE MUTATIONS ON RESPIRATORY CHARACTERISTICS OF MAIZE LEAF TISSUE[1,2]

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This report represents a preliminary survey comparing respiratory characteristics of mutant and normal corn plants from isogenic lines. Morphologically the same tissue was used for all comparisons and mutants and normal inbred seedlings were grown side by side under the same environmental conditions. Any differences which were detected presumably represented phenotypic expressions of single genes.

The genetic background of the corn plants employed in this survey was that of the University of Minnesota Agricultural Experiment Station Inbred, A 21. The mutant, dwarf-1 (1), was introduced into the inbred in 1940 and mutant progeny were backcrossed to the inbred five times. The mutant, brachytic-2 (3), was introduced in 1943 and homozygosity was similarly increased by backcrossing.

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Phenotypically the two mutants are morphologically dissimilar. Dwarf-1 is a seedling mutant which can be distinguished from normal siblings soon after germination. Leaves of the mutant are darker and broader. In stature the mature plant is a miniature of the normal attaining about one-third the height of the latter.

Brachytic-2 is indistinguishable from normal seedlings and can be identified only as the plants mature. Mutant plants attain about two-thirds the height of the normal.

These particular corn mutants have been the subject of anatomical and developmental studies in this department (2, 4, 6). The breeding program requisite to this research in developmental morphology provided the necessary seed from isogenic stocks and advantage was taken of the availability of this material to study differences in the corn mutants on a physiological rather than a morphological basis. In each experiment seed all from the same seed-lot was used to provide the experimental plants. In the case