VITAMIN K STIMULATION OF SPHINGOLIPID SYNTHESIS

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SUMMARY: The addition of vitamin K to a culture of Bacteroides melaninogenicus grown in a vitamin K free medium stimulated synthesis of the phosphosphingolipids but not other phospholipids such as phosphatidylethanolamine and phosphatidylserine. This stimulation of synthesis of the phosphosphingolipids occurs soon after the addition of the vitamin to the culture and before an increase in general cell metabolism as indicated by an increase in growth rate of the vitamin K supplemented culture.

Bacteroides melaninogenicus is a gram negative obligate anaerobe which has nutritional requirements for vitamin K, protoheme and peptides (1,2,3). This bacterium is unusual in that it contains several phosphosphingolipids such as ceramide phosphorylethanolamine (CPE) and ceramide phosphorylglycerol (CPG) as well as other phospholipids (4). Succinate can replace in part the requirement for vitamin K allowing serial growth of the microorganism in the absence of vitamin K (5). Using cells which have been serially subcultured in the absence of vitamin K, we have shown (6) that the supplementation of this culture with the vitamin stimulates synthesis of both CPE and CPG whereas other phospholipids, phosphatidylethanolamine (PE) and phosphatidylserine (PS) are unaffected by the addition of the vitamin.

In previous experiments cells were used which were deprived of vitamin K for many successive subcultures in the presence of succinate and so were stressed with regard to their vitamin K requirement. In this communication we wish to report experiments on the influence of vitamin K on sphingophospholipid synthesis in cells which were vitamin K depleted under less rigorous conditions by limited growth in a vitamin K free medium.

MATERIALS AND METHODS

The trypticase-yeast extract medium used to grow the microorganism and the conditions of growth have been described previously (3). Experiments were performed using the modified anaerobic jar (7) which allows samples to be injected and withdrawn from an anaerobic culture without disturbing the E_h. The strain of B. melaninogenicus used was originally derived from the bovine rumen and was used in previous experiments on vitamin K metabolism (3).

Vitamin K depleted cells were prepared by inoculating 2 ml of a 2 day culture grown in the presence of 0.1 μg/ml vitamin K_1 (Nutritional Biochemicals Co., St. Louis, Missouri) into 200 ml of medium containing laked horse red cells and incubated for 16-24 hrs. Growth (cell mass) under these conditions using a large inoculum is slow and linear and occurs to some degree as a result of cell elongation (3).

Samples of cells were centrifuged, washed once in phosphate buffer (0.05M, pH 7.0) and lipids extracted by the method of Bligh and Dyer (8). Lipids were analyzed by chromatography on silica gel C plates using chloroform, methanol, water 70:25:4 for the first direction and chloroform, methanol, 7M ammonia 60:35:4 for the second. Individual lipids were identified by cochromatography using authentic standards. The ceramides CPE and CPG were identified by their resistance to mild alkaline methanolysis and by their chromatographic mobilities according to the scheme of Rizza, Tucker and White (4). Lipids were located on plates with iodine vapors, scraped off and analyzed for phosphate by the method of Norton and Autilio (9). In other experiments spots were located by radioautography scraped off the plates and radioactivity counted.

The influence of vitamin K on the incorporation of 2-3 ^{14}C succinic acid into phospholipid was examined as follows: vitamin K depleted cultures were prepared and incubated for 16 hrs. Two cultures were chosen which had similar turbidity readings (approximately 140 Klett units using a red filter), 50 μc 2-3 ^{14}C succinic acid (New England Nuclear 5.84 mc/mM) and 0.1 μg/ml vitamin K_1 were added to one culture and the other control culture received 50 μc succinic

acid only. At 45, 90, 135 and 180 min. following the inoculation of succinic acid and vitamin K, 25 ml samples of culture were removed, the turbidities measured, the cells washed and lipids analyzed as described above. The incorporation of label into individual lipids was then determined.

RESULTS AND DISCUSSION

Fig. 1 shows the result of vitamin K supplementation of a vitamin K depleted culture on the incorporation of succinate carbon into CPE and CPG. Following the administration of vitamin K at time zero, there is an increased incorporation into both ceramides. Conversely, as shown in Fig. 2, the administration of vitamin K to a depleted culture has no effect on the incorporation of succinate into PE and PS. During the period of experiment, i.e., 180 min. following the administration of vitamin K and succinate, the turbidities of both cultures had increased by the same amount C. 24 Klett units. Following 180 min., the vitamin K supplemented culture showed a rate of growth greater than the control

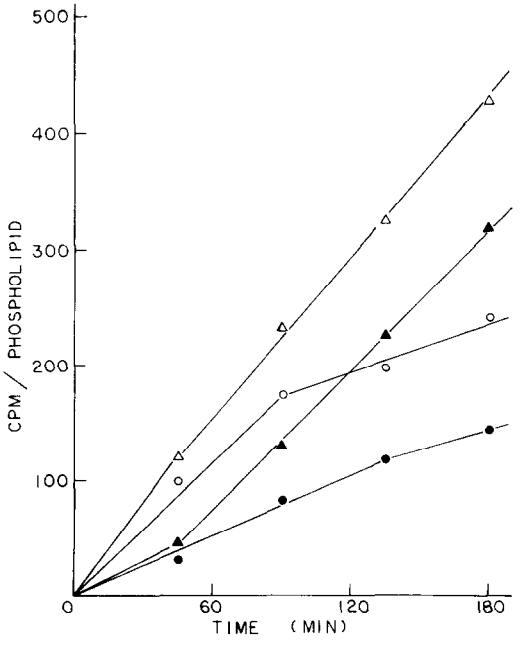

FIGURE 1: Influence of 0.1 μg/ml vitamin K_1 on the incorporation of 2-3 ^{14}C-succinate, both added at time zero, into (a) ceramide phosphorylethanolamine, culture plus vitamin K Δ, control culture (no vitamin K) O, and (b) ceramide phosphorylglycerol, culture plus vitamin K ▲, control culture ●.

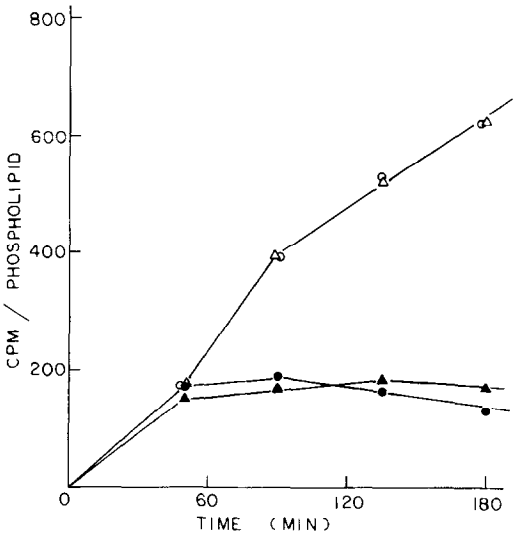

FIGURE 2: Lack of influence of vitamin K on the incorporation of 2-3 ^{14}C-succinate into (a) phosphatidylethanolamine, culture plus vitamin K Δ, control culture O, and (b) phosphatidylserine, culture plus vitamin K ▲, control culture ●.

culture. Phosphorous analyses on lipids from culture samples taken 135 min. after supplementation showed the following levels: PS 4.4, CPE 11.0, CPG 11.8, and PE 21.7 μM P/g cells for the vitamin K supplemented culture. The unsupplemented control culture showed PS 4.0, CPE 8.7, CPG 10.0, and PE 23.7 μM P/g cells. Thus, the increase in incorporation into CPE and CPG following the administration of vitamin K represents increased synthesis of these phosphosphingolipids.

During the period of experiment, the growth rate of both cultures was the same. This shows that the stimulation of synthesis of CPE and CPG occurs before an increase in general cell metabolism. Vitamin K did not influence the incorporation of succinate into PS and PE. Thus, following the administration of vitamin K to a depleted culture there is an early and specific effect only on the sphingolipids.

It was previously noted (6) that in vitamin K deficient cells lipid metabolism is deranged, i.e., the incorporation of succinate into lipids and phospholipids was greater in deficient cells compared to those grown with

vitamin K. This effect was found also with palmitate (unpublished observations). Thus, although we have shown a specific effect of vitamin K on sphingolipid synthesis, vitamin K would also appear to affect other aspects of lipid metabolism. These results of vitamin K stimulated sphingolipid synthesis found under much less rigorous conditions of vitamin K deficiency confirm those found when cells are grown continuously in the absence of vitamin K but in the presence of 2×10^{-2} M succinate.

The effect of vitamin K on the synthesis of cell envelope lipids are of interest in that this system presents an approach to the study of the synthesis of these compounds under perturbed conditions and hence may lead to the elucidation of the function of the complex phosphosphingolipids in the cell envelope.

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