EFFECTS OF THIAMINE AND PYRIDOXINE ON THE COMPOSITION OF FATTY ACIDS IN SACCHAROMYCES CARLSBERGENSIS 4228

Yoshiki Nishikawa, Ichiro Nakamura, Teijiro Kamihara and Saburo Fukui*

Laboratory of Industrial Biochemistry, Department of Industrial Chemistry, Faculty of Engineering, Kyoto University, Sakyo-Ku, Kyoto, Japan

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SUMMARY The cells of Saccharomyces carlsbergensis 4228 growing aerobically in the presence of thiamine and absence of pyridoxine, which were in a deficient state of respiratory activity, showed a marked decrease in the content of unsaturated fatty acids. Addition of pyridoxine to the medium prevented completely this effect of thiamine as observed in the case of respiratory activity.

In a preceding paper (1), we reported a marked decrease in the respiratory activity of Saccharomyces carlsbergensis 4228 (ATCC 9080) growing aerobically in the presence of thiamine and absence of pyridoxine—that is, decrease in the respiration rate (oxygen-uptake) and in cytochrome oxidase activity, and loss of the absorption spectra of cytochromes. It was also shown that addition of pyridoxine prevented these effects of thiamine. The present communication describes that thiamine and pyridoxine affected also the contents of unsaturated fatty acids of the yeast.

METHODS

Growth of yeast The cultivation of S. carlsbergensis 4228 (ATCC 9080) and growth measurement were carried out as described in the preceding paper (1). When indicated, the amount of Ca-pantothenate or biotin was limited to 2.5 ng or 0.016 ng per ml, respectively.

Extraction of fatty acids from cells Cellular fatty acids were extracted under reflux in 20 % methanolic KOH (w/v) for 3 hours at 70° C. After methanol had been evaporated, non-saponifiable materials were removed with petroleum ether. Fatty acids were extracted with diethyl ether in pH ranges below 2.

Fatty acid analysis Methyl esters of the extracted fatty acids were prepared with boron trifluoride in methanol. Gas-liquid chromatography (GLC) was carried out on a Yanagimoto 550T Gas Chromatograph equipped with a hydrogen flame ionization detector at 170° C with a flow rate of helium of 17-20 ml per min, by using a 0.3 X 225 cm stainless steel column of 15 % diethylene glycol succinate on 60-80 mesh Neopak AS (Nishio Kogyo Co., Japan). The column was

* To whom correspondence and reprint request should be addressed.

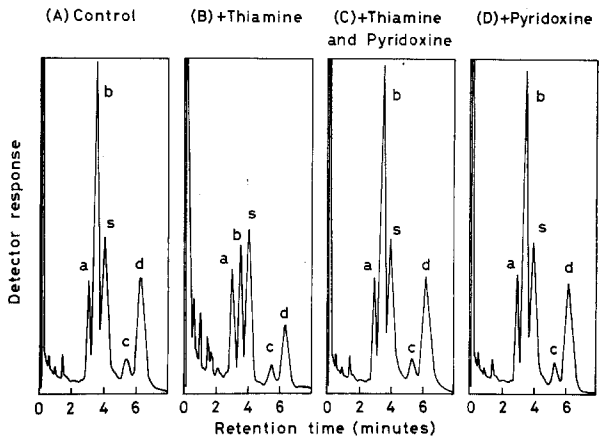

Fig. 1 Gas-liquid chromatography tracings of fatty acid methyl esters from S. carlsbergensis 4228 cells grown (A) without both thiamine and pyridoxine (B) with thiamine alone (C) with both thiamine and pyridoxine and (D) with pyridoxine alone. Individual methyl esters were separated as described in the text and marked as follows: (a) palmitate (b)palmitoleate (c) stearate (d) oleate, and (s) internal standard (heptadecanoate).

calibrated with a standard mixture of methyl esters of fatty acids. Peak area was determined by triangulation and the amount of fatty acid ester per g of dry cells was estimated from GLC tracings with heptadecanoic acid as internal standard.

RESULTS

Effects of thiamine and pyridoxine on cellular fatty acids

Figure 1 shows the results of GLC analysis of fatty acid esters in the cells of S. carlsbergensis harvested in middle logarithmic phase. The cells grown in the absence of both thiamine and pyridoxine contained mainly palmitoleic and oleic acids, and minor amounts of the corresponding saturated fatty acids, palmitic and stearic acids. A striking decrease was observed in the content of unsaturated fatty acids of the cells grown in the presence of thiamine alone, whereas that of the saturated fatty acids was slightly increased. The cells growing with pyridoxine exhibited the normal composition of fatty acids even in the presence of thiamine. These features were clearly demonstrated by the data shown in Table 1. The ratio of monounsaturated to saturated fatty acids was about 9:1 in control cells, whereas it decreased to about 2:1 in the cells grown with thiamine alone ("thiamine-cells"). Furthermore, the difference in the fatty acid content between control and "thiamine-cells" were not so much affected by the time of harvesting (data not shown).

Effects of pantothenic acid or biotin deficiency on cellular fatty acids

To evaluate the specificity of the changes in fatty acid composition in

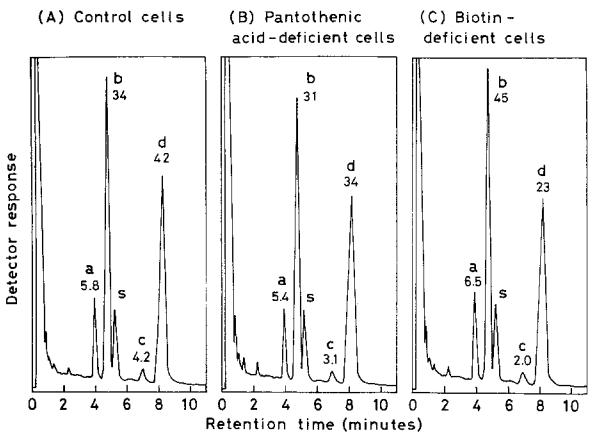

Fig. 2 Gas-liquid chromatography tracings of fatty acid methyl esters from (A) control (B) pantothenic acid-deficient (C) biotin-deficient cells of S. carlsbergensis 4228. Individual fatty acid contents (mg/g-dry cells) are indicated below the identifying letters. The determination of the content was carried out as described in the text. Symbols for individual fatty acid methyl esters are the same as those in Fig. 1.

"thiamine-cells", GLC analyses were performed on the same microorganism cultured on growth-limiting amounts of Ca-pantothenate or biotin and harvested in the middle exponential phase of growth. Deficiency of each vitamin lowered the growth rate as well as the cell yield. Both pantothenic acid deficiency and biotin deficiency altered the fatty acid composition of the yeast cells slightly. Neither produced the drastic decrease in palmitoleic and oleic acids, which was characteristic of "thiamine-cells" (Figure 2). These results indicated that the marked decrease in the unsaturated fatty acid content would not be a general phenomenon in growth depression, but would be resulted from a specific action of thiamine on the yeast.

DISCUSSION

In 1965, Haskell and Snell (2) reported that a dramatic decrease occurred in the palmitoleic acid content of the cells of Hanseniaspora valbyensis grown in a vitamin B_6-deficient medium. As described in our preceding paper (1), we observed a marked decrease in the vitamin B_6 content of the cells of S. carlsbergensis 4228 grown in the presence of thiamine and absence of pyridoxine. These facts strongly suggest that the primary event of the thiamine action in S. carlsbergensis may be the vitamin B_6 deficiency which would result in the lowering of the unsaturated fatty acid content of the cells. As we discussed in the preceding paper (1), the vitamin B_6 deficiency caused by thiamine might be associated with the decrease of cytochromes. This cytochrome

Table 1 Effects of thiamine and pyridoxine on the contents* of saturated and unsaturated fatty acids (C_{16} and C_{18}) in the cells of S. carlsbergensis 4228

Cells	Contents of fatty acids (mg/g-cells)	
	Palmitic acid + Stearic acid	Palmitoleic acid + Oleic acid
Control	8.14	78.9
+ Thiamine	9.94	20.9
+ Thiamine + Pyridoxine	8.27	71.7
+ Pyridoxine	8.17	66.7

* The C_{16} and C_{18} fatty acids were selected, since these were the main components of fatty acids in the cell as shown in Fig. 1.

deficiency in the vitamin B_6-deficient cells would induce the decrease in the unsaturated fatty acid content. The desaturation of long chain fatty acids is known to occur by the reaction catalyzed by cytochrome b_5 in rat liver (3). In yeast, a similar mechanism has been proposed (4).

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