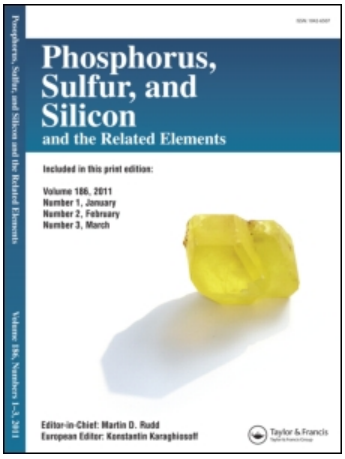

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P-31 NMR STUDY OF POLYPHOSPHATES IN YEAST CELLS

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Abstract It was found that the chain length of polyphosphates (poly P), which are presented in various cell compartments, depends strongly on the growth stage and cultivation conditions of yeast cells. It was newly revealed, that only the chain length of poly P increased, while the amount of poly P remained constant during the poly P biosynthesis in cells.

Key Words: P-31 NMR study on polyphosphates, yeasts.

INTRODUCTION

Numerous studies on the structure and properties of polyphosphatases and polyphosphatkinases, in fact, contain no information about depolymerases, that cleave poly P to more short groups, which, in turns, are substrates to polyphosphate-specific enzymes [1]. As possible criteria of the enzyme activity, quantitative and qualitative levels of poly P in a cell may be estimated by P-31 NMR-spectroscopy.

In this work, we studied some specific features of the poly P accumulation and assumed that the chain length of poly P was dependent on the growth stage of culture.

MATERIALS & METHODS

P-31 NMR-spectra were recorded on a 'Bruker' (AM-400) instrument, D_20 was added for lock-signal.

To isolate fractions of poly P, Saccharomyces cerevisiae (BKM Y-1173) cells were treated as described in [2]; five poly P fractions were obtained. The cell culture were grown in phosphate-free and phosphate-containing media.

Samples were collected on the 4, 11, 13, and 15 hours of the culture growth.

The chain length (n) was estimated for each fraction over the culture growth. Poly P chain length was determined by the ratio of intensities of signals attributed to the central, preterminal, and terminal residues in the poly P molecule.

RESULTS & DISCUSSION

We varied cultivation conditions that manifested themselves in changing amount and chain length of each poly P fraction (Fig. 1-3). Fig. 1 shows that, after replacement of the yeast cells from a complete medium (point A) on a phosphate-free medium, the cells continued to grow and their biomass increased 4-fold over 7 h (point B); while, poly P was, in fact, completely consumed. Within 7 h of starvation, only 5% of all poly P were found in cells. After further replacement of the yeast culture to a complete phosphate-containing medium, the rapid accumulation of poly P (phenomenon of 'overcompensation') was observed, and the total poly P content increased more than two-times after 2 h (point C). Table 1 shows that, the poly P fractions 3 and 4 are maximally accumulated and content rose 4.5 and 8.5-fold, respectively, as compared to that at the point A.

Experimental data suggests that, under various cultivation conditions, the different poly P fractions are not uniformly consumed, or accumulated due various compartmentalization within the cell; and therefore, each poly P fraction has its own pathway of biosynthesis (Table 1).

In the case of phosphate starvation (point A), a mostly significant decrease in the poly P chain length was observed for the poly P fractions 3 and 4, unlike the poly P fraction 2, for which a sharp increase in the chain length was observed (Fig. 3). Note,

TABLE 1.
Quantitative changes in polyP fractions during different cultivation conditions.
The content of polyP fraction in the culture (point A) is taken as 100 %.

poly P fractions	Cultivation conditions			
	Complete medium (+P), 4 hours	Starvation (-P), 7 hours	Complete medium (+P), 2 hours	Complete medium (+P), 4 hours
	A	B	C	D
$HClO_4$, 0°C	100	2.4	138.6	121.1
$NaClO_4$, 0°C	100	5.3	206.6	143.0
NaOH, pH 8-9, 0°C	100	1.18	465.5	464.1
NaOH, pH 12, 0°C	100	9.1	82.2	97.4
$HClO_4$, 90°C	100	48.8	851.8	1235
Σ poly P	100	4.8	223.3	211.1

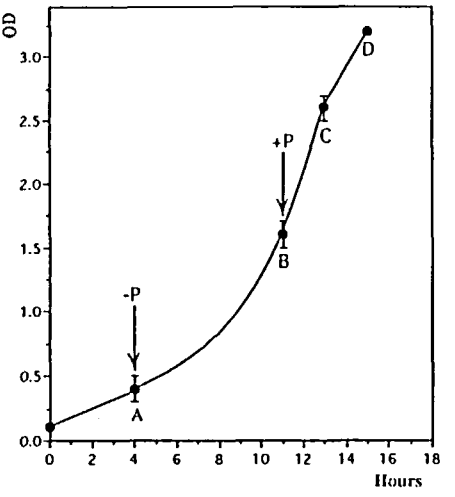

FIGURE 1 Biomass growth.

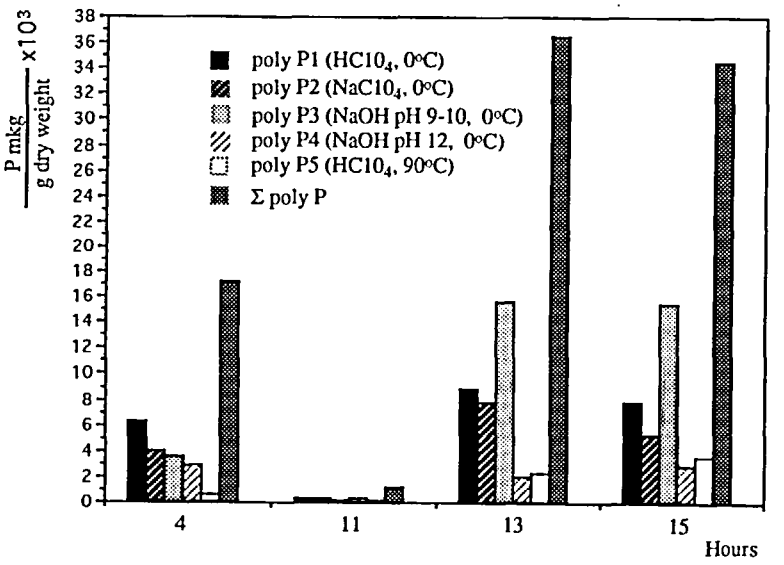

FIGURE 2 Accumulation of poly P fractions during culture growth.

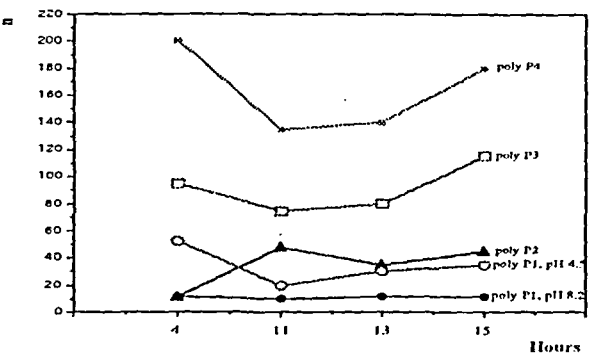

FIGURE 3 Changes in the chain length during cell cultivation.

within 2 h of the culture growth in the phosphate-containing medium, the chain length remained constant; whereas, their content sharply increased. Within 7 h of the cultivation (point B), the chain length began to grow, except the poly P fraction 2, whose chain length rose only within 4 h of the culture growth (point C).

Thus, the 'overcompensation' phenomenon involves two stages; the first one is followed by significantly increasing of short-length poly P amount; while, at the second stage the amount of long-chain poly P increased. The data obtained are considered with respect to the action of enzymes responsible for synthesis and hydrolysis of poly P. Likely, within the first hours of overcompensation, the accumulation (or activity) of polyphosphate-synthesizing enzymes and the consumption of poly P proceed simultaneously, yielding the short-length poly P. Then, after the culture growth in a phosphate-containing medium, when the enzyme biosynthesis sharply lowered, the poly P chain length increased, without considerable accumulation of poly P. To confirm this assumption, further experiments must be performed, because the synthesis of polyphosphatases is known to be not induced by phosphate starvation of some fungi [3].

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