THE EFFECT OF AMINO ACIDS ON GROWTH AND PHOSPHATE METABOLISM IN A PROTOTROPHIC YEAST STRAIN

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Received September 21,1977

SUMMARY

The addition of casamino acids to a log. phase culture of a prototrophic yeast strain under conditions in which their catabolism is repressed caused stimulation in growth rate. The neutral amino acids and arginine were the principal contributors to this stimulation effect. An early response of the cells to the addition of amino acids was the accumulation of low molecular weight polyphosphates. This accumulation was shown to correlate to the basicity of a given amino acid rather than to its effect on growth rate. A role for the polyphosphates in intracellular buffering is therefore suggested.

INTRODUCTION

During the course of our studies on the coordination of transcription with translation in the yeast, Saccharomyces cerevisiae, we have investigated the effect of adding amino acids to a culture of a prototrophic strain growing under conditions of ammonium repression. Previous workers (1,2) have reported experiments on amino acid step-up of yeast growing under conditions of nitrogen limitation and the efficiency of various amino acids as nitrogen sources for yeast has been determined (3). In this communication, we report the effects of various combinations of amino acids on the growth of prototrophic yeast when added to a nitrogen sufficient medium. The effect of these amino acids on the formation of low molecular weight polyphosphates is also demonstrated. The possible physiological role of the latter compounds is discussed.

ISSN 0006-291X

TABLE I

EFFECT OF AMINO ACID MIXTURES ON GROWTH

Addition to medium	Growth rate doubl./hr.	Percent of casamino acid step	Step point min.
None (control)	0.337	-	-
Vitamin-free casamino acids (2 mg/ml)	0.405	100	118
'Cas-like' amino acids (2 mg/ml)	0.400	93	100
20 L-amino acids (each at 0.25 mM)	0.394	83	100
19 L-amino acids (tyr omitted) (each at 0.25 mM)	0.406	101	130
Basic amino acids (0.25 mM each of arg, his, lys)	0.372	51	120
Neutral amino acids (0.25 mM each of ala, phe, ile, val, pro, thr, ser, gly, leu)	0.369	47	120
Acidic amino acids (0.25 mM each of asp, glu)	0.342	8	120
Remaining amino acids (0.25 mM each of cys, met, gln, asn, trp)	0.354	25	120
Arginine (0.25 mM)	0.360	34	120
Tyrosine (0.25 mM)	0.332	-7.7	-
Lysine (0.25 mM)	0.340	3.9	-
Glutamine (0.25 mM)	0.337	<1	-
NH_4Cl (10.0 mM)	0.337	<1	-

MATERIALS AND METHODS

Organism: Prototrophic, haploid strain S7 of Saccharomyces cerevisiae has been described previously (4).
Media: The defined medium (YNB) used in all experiments consisted of 0.67% Yeast Nitrogen Base Without Amino Acids (5) plus 2%

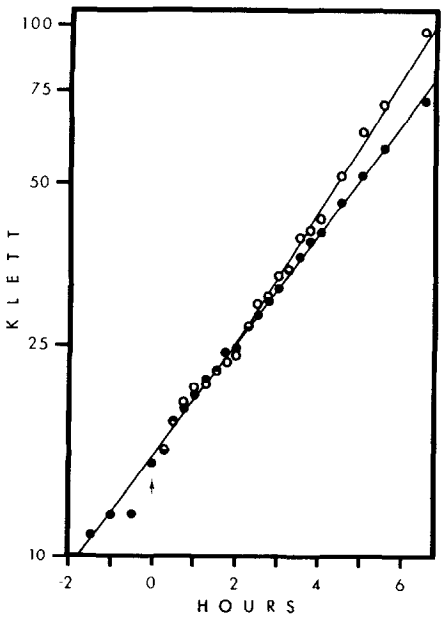

Figure 1: Effect on growth of adding vitamin-free casamino acids (2 mg/ml) to a log. phase culture of S7. ●—●, control; o—o, + casamino acids.

glucose. Where indicated, this medium was buffered to pH 5 with 1% succinic acid and 0.6% NaOH. Vitamin-free casamino acids (Difco) and L-amino acids solutions were filter sterilized before adding to this medium.

Growth: Cultures were shaken at 23°C in Nephlo flasks (Bellco). Growth was monitored with a Klett-Summerson colorimeter using a green filter.

Assay of acid-soluble polyphosphates: Log. phase cells were prelabelled for 2 hr. with ^{32}P-orthophosphate (carrier-free; Amersham) at a concentration of 100 µCi/ml. Radioactive phosphate was present throughout the ensuing experiment. Samples (40 µl) of the cultures were taken into an equal volume of ice-cold 2N formic acid and extracted for at least 30 min. The cells were centrifuged out and aliquots (10 µl) of the extract spotted onto polyethyleneimine (PEI) - cellulose thin-layer plates. These plates were developed in one-dimension with 1.5 M KH_2PO_4, pH 3.4 (6). After drying, the position of radioactive spots was determined by autoradiography using BB54 X-ray film (Kodak). Spots were cut out and radioactivity measured by liquid scintillation counting in a toluene-based fluid (7).

RESULTS AND DISCUSSION

Medium YNB contains 5 mg/ml $(NH_4)_2SO_4$ and catabolism of amino acids is therefore repressed (8). The effect of adding various

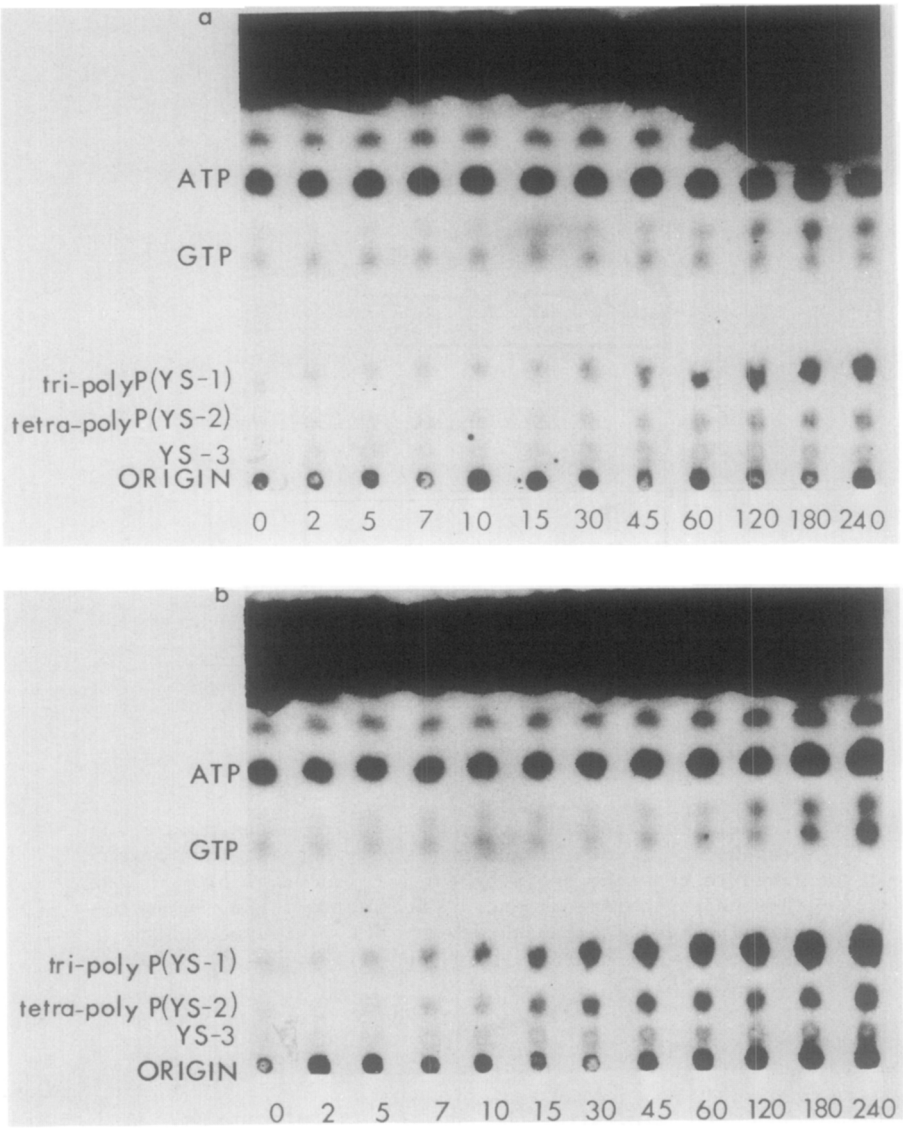

Figure 2: Autoradiographs of PEI - cellulose plates showing accumulation of polyphosphates by S7 growing under control conditions (a) and during step-up with vitamin-free casamino acids (b).

combinations of amino acids to a log. phase cultures of S7 in buffered YNB is summarized in Table 1. Figure 1 shows a representative growth curve and demonstrates the effect of a casamino acids step-up. It can be seen that there is an abrupt change in

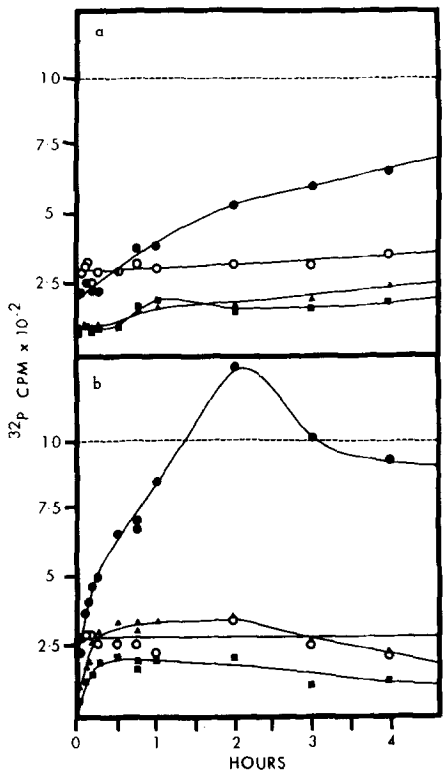

Figure 3: Accumulation of tripolyphosphate during a casamino acids step-up. ^{32}P counts/min. in tripolyphosphate are normalized to constant activity for ATP (shown as broken line) throughout the experiment. 3A, Control; 3B, +2 mg/ml vitamin-free casamino acids. ●—● , tripolyphosphate (YS1); ▲—▲ , tetrapolyphosphate (YS2); ■—■ , YS3; ○—○ , GTP.

growth rate about 120 min. after the addition of amino acids. We call this point the 'step point'. It can be seen from Table 1 that the step point occurs at approximately the same interval for all amino acid additions. This interval probably reflects a constant period in the yeast cell cycle (9). It can be concluded that the amino acids did not increase growth rate by acting as an additional source of nitrogen since glutamine, the most favourable nitrogen source, had little or no effect on growth rate. Moreover, adding more nitrogen (as NH_4Cl, 0.535 mg/ml) did not stimulate

TABLE 2

RELATIVE ACCUMULATION OF TRIPOLYPHOSPHATE

Addition to medium	Tripolyphosphate accumulated in 2 hr. relative to control
None (control)	1.00
Neutral amino acids (see Table 1; total conc. 5.0 mM)	0.95
Basic amino acids (see Table 1; total conc. 5.0 mM)	4.66
Arginine (0.25 mM)	2.71
Lysine (0.25 mM)	2.07
Alanine (1.0 mM)	0.804
Aspartic acid (1.0 mM)	0.858

growth. Table 1 shows that the major contribution to the increase in growth rate came from the basic and neutral amino acids. The largest single contribution came from arginine. This amino acid has one of the longest biosynthetic pathways and yeast carries a large intracellular arginine pool (10).

In an attempt to determine what triggers the increase in macromolecular synthesis which follows an amino acid step-up (11), the formation of low molecular weight polyphosphates was investigated. These compounds have been characterized in yeast by Lusby and McLaughlin (12). High molecular weight polyphosphates were found to inhibit RNA synthesis in Physarum (13). The conversion of high molecular weight polyphosphates to their low molecular weight derivatives might therefore trigger the increase in RNA synthetic rate which occurs about 20 min. following the addition of amino acids to a log. phase culture of yeast (11).

Figure 2 shows the autoradiographs produced by the ion-

exchange chromatography of a formic acid extract from yeast during a casamino acids step-up. Spots were identified in accordance with Lusby and McLaughlin (12). Quantitative data from these autoradiographs are given in Figure 3. It can be seen that there was an immediate increase in concentration of tripolyphosphate, tetrapolyphosphate, and a third, more slowly migrating, species (YS3) on adding amino acids to the medium. This increase preceded any increase in the rate of RNA synthesis (11). Thus, the kinetics of low molecular weight polyphosphate formation were compatible with these compounds being signal molecules which trigger the increase in the rate of macromolecular synthesis.

We next attempted to determine whether there was any correlation between the ability of an amino acid (or a group of amino acids) to promote the accumulation of tripolyphosphate and its ability to stimulate growth. The data (Table 2) indicates that while the basic amino acids produce almost a 5-fold stimulation in the formation of tripolyphosphate, the neutral amino acids have no effect. However, it was shown (Table 1) that the basic and neutral amino acids each contributed about half of the growth stimulation produced by a complete amino acids mixture. It therefore appears that tripolyphosphate is more likely to act as a cellular buffer, neutralizing the incoming basic amino acids, than as a regulatory signal. This idea is confirmed by studies of the effect of individual amino acids (Table 2). Both arginine and lysine increase the accumulation of tripolyphosphate but only arginine stimulates growth. The effect of these two basic amino acids contrasts with that of the neutral alanine and acidic aspartate, neither of which increase the concentration of tripolyphosphate. It must therefore be concluded that if tripolyphosphate

has a regulatory role in addition to its buffering function, it must be rather specific in its effect.

ACKNOWLEDGEMENTS

This work was supported by an N.I.H. grant (CA10628) to C.S.M.. S.G.O. was supported in part by an S.R.C. Fellowship. We thank Dr. E.W. Lusby, Jr. for his introduction to the polyphosphates and Dr. Francine Messenguy for helpful discussions. J.R.L. thanks Professor K.A. Stacey for the hospitality of the Canterbury Lab.

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