REGULATION OF PTEROYLGLUTAMATE POOL SIZE BY METHIONINE IN *SACCHAROMYCES CEREVISIAE*

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1. Introduction

The biogenesis of methyl groups in certain bacteria [1–4] and animal tissues [5–7] is now known to be regulated by methionine. This amino acid appears to exert its effect by repression of 5,10-CH_2-H_4PteGlu** reductase in *Escherichia coli* [2] and by inhibition of this enzyme in mammalian liver [7]. In animals, administration of methionine results in decreases in 5-CH_3-H_4PteGlu [8] but to our knowledge no such studies have been reported for yeasts grown in defined media. In *Saccharomyces*, it is clear [9] that methyl groups required for the biosynthesis of methionine can arise from serine. Furthermore, methionine and *S*-adenosylmethionine were found to inhibit the activity of serine hydroxymethyltransferase. Although production of one-carbon units from serine could be regulated by these compounds in vivo, the physiological significance of this is not clear particularly if serine is an important precursor of thymidylate.

The present studies have shown that levels of pteroylglutamate derivatives in yeast cells grown in media devoid of methylated compounds are markedly decreased by supplementing the media with L-methionine. Although all components of the pteroylglutamate pool were decreased by this supplement, the greatest reductions occurred in the levels of methylated pteroylglutamates. Extracts of cells grown under these conditions contained similar levels of four enzymes catalyzing synthesis and interconversion of the principal pteroylglutamates found in the cells. The activity of 5,10-CH_2-H_4PteGlu reductase was markedly inhibited by additions of L-methionine to the reaction system. It is suggested that methionine regulates the biosynthesis of one-carbon units for methionine formation in yeast by its inhibitory effect on this reductase.

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** The abbreviations used for pteroylglutamic acid and its derivatives are those suggested by the IUPAC-IUB Comission as listed in Biochem. J. 102 (1967) 15: e.g. 10-HCO-H_4PteGlu = N^{10}-formyltetrahydropteroylmonoglutamate.

2. Materials and methods

2.1. *Culture conditions*

Saccharomyces cerevisiae (ATCC 9763) was grown in a complete defined medium (medium A) or a minimal medium (medium B). Medium A contained (per 100 ml): 50 μg thiamine, 5 mg inositol, 500 μg each of calcium pantothenate and niacin, 2 μg biotin, 85 mg KCl, 1.5 mg $MgSO_4 \cdot 7H_2O$, 500 μg each of $FeCl_3 \cdot 6H_2O$ and $MnSO_4 \cdot H_2O$, 25 mg $CaCl_2 \cdot 2H_2O$, 110 mg KH_2PO_4, 200 mg $(NH_4)_2HPO_4$, 10 g glucose and 40 mg each of DL-leucine, DL-valine, L-cystine, DL-phenylalanine, DL-threonine, DL-alanine, L-aspartic acid, L-lysine HCl and DL-serine, 50 mg L-glutamic acid, 20 mg each of L-isoleucine, L-tryptophan, L-tyrosine, L-arginine HCl, L-histidine $HCl \cdot H_2O$ and glycine, 10 mg L-proline. Medium B contained the above components with the exception of the amino acids and 4 g sucrose replaced glucose.

In all work, *S. cerevisiae* was subcultured aerobically at 30° for 24 hr in medium A supplemented with 2.5 μmoles/ml L-methionine, or in medium B. Cells from this culture were used to inoculate medium A or B, in the presence or absence of methionine. Cells

were grown for the desired length of time at 30° with aeration.

2.2. *Preparation of cell-free extracts*

After centrifugation, the cells were washed three times with cold sterilized distilled water, suspended in 1% K ascorbate and lyophilized.

H_4PteGlu derivatives were extracted after sonication in 0.6% K ascorbate solution (pH 6.0) and heating at 95° for 10 min [10]. After centrifugation, aliquots of the extracts were chromatographed on columns of DEAE-cellulose [11]. Fractions (3 ml) of the column effluent were collected and assayed for pteroylglutamate derivatives [12] using *Lactobacillus casei* (ATCC 7469) and *Pediococcus cerevisiae* (ATCC 8081). Lactic acid produced after 70 hr incubation at 37° was titrated as a measure of bacterial growth. The amounts of H_4PteGlu derivatives in each fraction and in the crude extract were calculated using reference curves [13]. Individual derivatives were identified by co-chromatography with authentic samples and by using γ-glutamyl carboxypeptidases from chicken pancreas and pea cotyledons.

2.3. *Enzyme assays*

Samples of the lyophilized material (10 mg) were suspended in 4 ml of 0.06 M phosphate buffer containing 1 mM EDTA and 2.8 mM ascorbic acid (pH 7.4). After centrifugation for 20 min at 3,000 *g*, the supernatant was retained for subsequent studies. 5,10-CH_2-H_4PteGlu dehydrogenase was measured as described by Ramasastri et al. [14], 10-HCO-H_4PteGlu synthetase by the method of Hiatt [15] and serine hydroxymethyltransferase according to Taylor and Weissbach [16]. 5,10-CH_2-H_4PteGlu reductase was assayed according to Dickerman and Weissbach [17]. Protein was determined colorimetrically [18].

3. Results

3.1. *Effects of L-methionine on levels of H_4PteGlu derivatives during growth*

The pteroylglutamate levels of yeast cultures reached maximal values at an early stage of logarithmic growth (fig. 1). The highest contents were found in cells cultured in the absence of methionine. Addition of this amino acid to both media resulted in a 50–60%

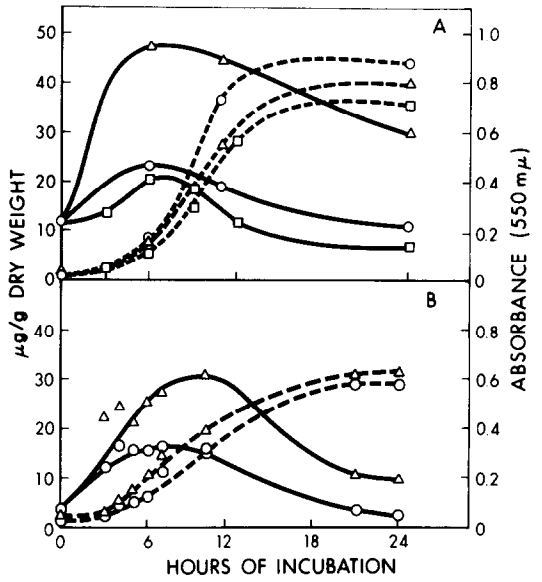

Fig. 1. Changes in pteroylglutamate pool size during growth. (A and B) Cells were cultured in medium A and medium B respectively. Absorbance at 550 mμ (dotted lines) and pteroylglutamate levels (solid lines) were determined for cultures without methionine supplement (△) and with supplements of 2.5 μmoles/ml (○) and 5 μmoles/ml (□) of L-methionine.

decrease in the total pteroylglutamates assayed before γ-glutamyl carboxypeptidase treatment. Incubation of the yeast extracts with chicken pancreas carboxypeptidase increased the levels of these derivatives by approximately 3 fold, however, the methionine grown cells still contained only 40–50% of the total levels found in extracts of cells grown in the absence of this amino acid.

The elution and identification of the individual pteroylglutamates in these extracts are shown in fig. 2. Several derivatives were present including 10-HCO-H_4PteGlu, 5-CH_3-H_4PteGlu, H_4PteGlu, and 5-CH_3-H_4PteGlu$_2$. In addition a derivative eluting at a position corresponding to that of 5-CH_3-H_4PteGlu$_3$ was conspicuous in extracts of cells grown in the absence of methionine. In extracts of cells grown in the presence of methionine the levels of 5-CH_3-H_4PteGlu, 5-CH_3-H_4PteGlu$_2$ and the peak corresponding in position to 5-CH_3-H_4PteGlu$_3$ were drastically reduced. Smaller decreases in level were also observed for 10-HCO-H_4PteGlu and the

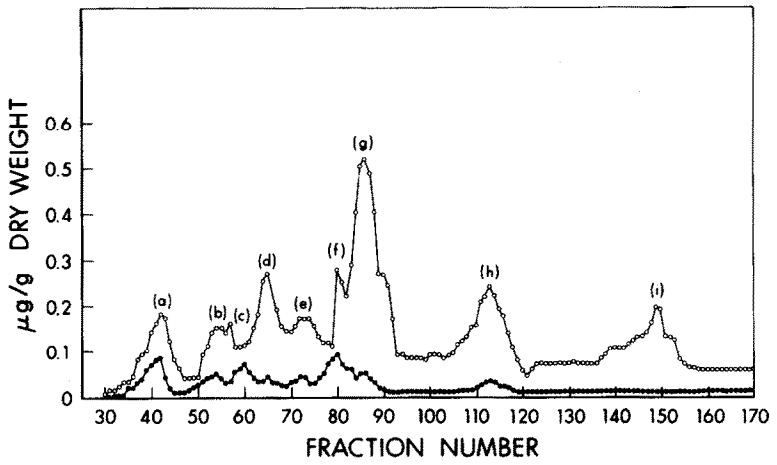

Fig. 2. Chromatography of pteroylglutamate derivatives. Extracts of cells grown for 24 hr in medium A without methionine supplement (○) and in medium A containing 2.5 μmoles/ml L-methionine (●) were subjected to chromatography on DEAE-cellulose. Peaks are identified as:
(a) 10-HCO-H_4PteGlu; (b) 10-HCO-H_4PteGlu$_2$; (c) 5-HCO-H_4PteGlu; (d) 5-CH_3-H_4PteGlu; (e) H_4PteGlu; (f) 5-HCO-H_4PteGlu$_2$; (g) 5-CH_3-H_4PteGlu$_2$; (h) 5-CH_3-H_4PteGlu$_3$; (i) unidentified conjugated derivatives.

remaining derivatives when methionine was supplied. Carboxypeptidase treatment of the extracts before chromatography resulted in large increases in the levels of formyl derivatives but did not appreciably change the levels of 5-CH_3-H_4PteGlu and 5-CH_3-H_4PteGlu$_2$. In the methionine grown cells the formyl compounds which occurred as polyglutamyl derivatives were greatly decreased in amount. Chromatography of extracts prepared from cells grown on medium B gave a similar pattern of pteroylglutamate derivatives and furthermore when methionine supplemented this medium the levels of methyl derivatives were again greatly reduced.

3.2. *Enzyme studies*

The specific activities of four enzymes of pteroylglutamate metabolism were determined during the logarithmic phase of growth on medium B with and without methionine supplement (table 1). Although some differences were observed in the specific activities of these enzymes, these were in general of a lower magnitude than normally associated with enzyme repression. The reductase was, however, readily inhibited by methionine when levels of this amino acid were included in the reaction system (fig. 3).

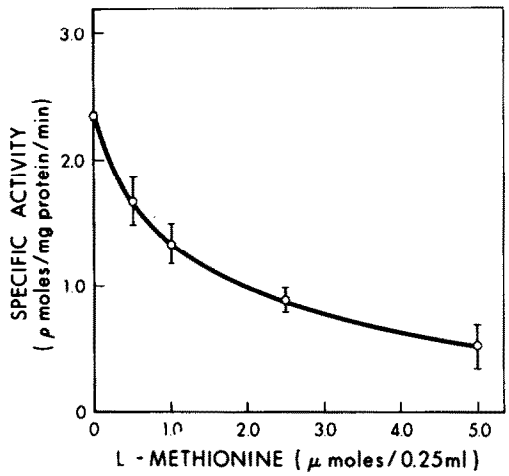

Fig. 3. Inhibition of 5,10-methylenetetrahydropteroylglutamate reductase by L-methionine.

Table 1
Specific activities of pteroylglutamate enzymes during logarithmic growth.

Enzyme	Medium B with L-methionine (2.5 mM)			Medium B without L-methionine supplement		
	Period of growth at 30° (hr)					
	4	7	10	4	7	10
5,10-CH_2-H_4PteGlu dehydrogenase*	0.97	0.60	0.67	1.29	0.72	0.42
10-HCO-H_4PteGlu synthetase*	0.27	0.41	0.32	0.25	0.71	0.54
Serine hydroxymethyltransferase**	1,880	3,340	2,840	1,100	2,480	2,660
5,10-CH_2-H_4PteGlu reductase**	1,300	1,720	1,880	920	2,060	1,360

* Product formed, μmoles/mg protein/hr.
** Product formed, dpm/mg protein/min.

4. Discussion

The decreases in the pteroylglutamate pool associated with methionine feeding could conceivably be due to either greater utilization of pteroylglutamates or a decreased production of these derivatives in the presence of exogenous methionine. The first possibility appears unlikely as earlier work by Botsford and Parks [9] has clearly shown that additions of methionine significantly reduced the biosynthesis of methionine from serine. Thus a decreased flow of one-carbon units through the methyl pteroylglutamate pool would be expected to occur in the presence of methionine. However, inhibition of serine hydroxymethyltransferase by methionine [9] cannot solely account for the decreases in pteroylglutamates observed in the present work as blockage of this reaction should lead to increases in the levels of H_4PteGlu. The dramatic decrease in the levels of methyl pteroylglutamates in the presence of methionine suggests that methyl group synthesis was to some extent selectively regulated by methionine. The absence of any large effect on the specific activities of the folate enzymes examined suggests that such regulation is not achieved by enzyme repression as is documented for *E. coli* [2]. The sensitivity of 5,10-CH_2-H_4PteGlu reductase to methionine (fig. 3) is similar to that reported for the mammalian enzyme and may, in *Saccharomyces*, play a physiological role in controlling methyl group biosynthesis. The above considerations do not however account for the overall decreases in pteroylglutamates which occurred when the cells were cultured in the presence of exogenous methionine. It is conceivable that this amino acid or one of its metabolic products directly or indirectly affected the net biosynthesis of pteroylglutamates. These possibilities are currently being investigated.

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