Methionine Metabolism of the Myxomycete *Physarum polycephalum*

JOHN W. DANIEL¹ AND KARLEE BABCOCK

McArdle Laboratory, University of Wisconsin, Madison, Wisconsin

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ABSTRACT

DANIEL, JOHN W. (University of Wisconsin, Madison), AND KARLEE BABCOCK. Methionine metabolism of the myxomycete *Physarum polycephalum*. J. Bacteriol. 92:1028-1035. 1966.—Previous studies have shown that *Physarum polycephalum* requires exogenous methionine for growth, but not cysteine, folic acid, or vitamin B₁₂. Methionine can also serve as the sole source of sulfur for all cellular requirements, without limiting the growth rate. *S*-methyl-L-cysteine, 2-hydroxy-4-methiol butyric acid, *S*-adenosyl-L-methionine, and methionine peptides were the only compounds supporting growth, when substituted for methionine. Other methionine analogues, methyl donors in combination with homocysteine, and intermediates of the cystathionine pathway were not active. Ethionine and *S*-ethyl cysteine were good methionine antagonists. This myxomycete is apparently unable to synthesize the methyl or *S*-methyl group, although it still appears able to transmethylyate, at least from *S*-methyl cysteine, and probably from *S*-adenosyl methionine, which can also serve as a source of adenine.

In a previous study (3), methionine was the only absolute amino acid requirement for the growth of *Physarum polycephalum* in submerged culture on a minimal synthetic medium. L-Methionine at 0.86 mM and the D isomer supported optimal growth. To locate the defect in the biosynthetic pathway, precursors and analogues of methionine were tested for their ability to replace or to antagonize methionine.

MATERIALS AND METHODS

Growth conditions, media, and cultures have been described in detail (3). The AV-40 medium contained 128 mg per liter of DL-methionine and 502 mg per liter of L-cysteine·HCl·H₂O. Growth was measured by determining the protein content of acetone-trichloroacetic acid-precipitated microplasmidia (3), with the method of Lowry et al. (12). All tests were performed with duplicate cultures. Compounds showing methionine activity were tested in two or more successive culture transfers. [The following corrections in the composition of the AV-40 medium should be noted in the published formula (Daniel et al, Bacteriol Proc., p. 53, 1962): biotin and thiamine are prepared as separate 50 times concentrated solutions (thiamine·HCl, 2.12 mg/100 ml; biotin, 7.9 µg/100 ml), and, as in Table 1, biotin is 1.58 µg per liter and DL-methionine, 128 mg per liter. In the present paper L-

methionine (128 mg per liter) replaces DL-methionine. The following corrections in the OV-40 medium amino acid concentrations should be noted: DL-methionine, 193 mg per liter; glycine, 680 mg per liter; and L-arginine·HCl, 908 mg per liter. These corrections make little difference in the growth rate obtained on the AV-40 medium (3), but they will increase somewhat the growth rates obtained on the OV-40 medium.]

L-methionine, DL-homocysteine, DL-homocysteine thiolactone, DL-α-amino-*n*-butyric acid, *S*-ethyl-L-cysteine, L-ethionine, choline, betaine, vitamin B₁₂, and folic acid were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio; DL-homoserine, sarcosine, L-methionine-DL-sulfoxide, and L-methionine sulfone, from Sigma Chemical Co., St. Louis, Mo.; DL-(+)-allo-cystathionine, dimethyl acetothetin, dimethyl-β-propiothetin, creatine, 2-hydroxy-4-methiol butyric acid, and *S*-adenosyl-L-methionine (SAM), from Calbiochem; L-(+)-*meso*-lanthionine, *S*-methyl-L-cysteine, and L-methionine, from Mann Research Laboratories New York, N.Y.; methyl mercaptan from Eastman Chemical Products, Inc., Kingsport, Tenn.; adenosine, from Pabst Laboratories, Milwaukee, Wis.

Aqueous solutions of test compounds were sterilized by filtration through a membrane filter (type HA; pore size 0.5 µ; Millipore Filter Corp., Bedford, Mass.). L-Methionine was usually autoclaved in the synthetic medium (15 psi, 20 min) and was also tested after sterilization by filtration. Media were used within 24 hr of sterilization; all additions were made just prior to inoculation.

¹ Present address: Johnson Research Foundation, School of Medicine, University of Pennsylvania, Philadelphia.

RESULTS

The methionine concentrations required for 50% growth and for maximal growth yield on several media are summarized in Table 1. The concentration required to obtain 50% maximal growth on various media was lower for lower growth rates (i.e., without glycine, and OV-40; Table 1), and was probably related to the greater sensitivity to methionine inhibition (3).

When substituted for methionine in the AV-40 medium, the following members of the cystathionine pathway and related compounds did not support growth of *P. polycephalum*: DL-homocysteine (7% of methionine activity), DL-homoserine (0%), DL-(+)-allocystathionine (0%), L-(+)-mesolanthionine (2%), DL-homocysteine thiolactone (2%), DL- α -amino-*n*-butyric acid + methyl mercaptan (14%), DL-homoserine + methyl mercaptan (8%), and no addition (0%). All compounds were tested at 0.86 mM, except methyl mercaptan was tested at 0.73 mM. L-Methionine allowed growth equivalent to 2.1 mg of protein per ml (100%), corrected for inoculum, after 4 days of growth. Other values are maximal for approximately 6 days of growth. Vitamin B₁₂ and folic acid, found nonessential in previous tests (3), did not stimulate growth. Normal growth occurred when the compounds were tested in combination with 0.86 mM L-methionine, the optimal concentration for growth on the AV-40 medium (3).

A number of *N*- and *S*-methyl compounds

were tested alone and in combination with homocysteine (Table 2). The presence of vitamin B₁₂ and folic acid did not alter the growth activity of these methyl donors. Only *S*-methyl-L-cysteine, which supported approximately equal growth with or without homocysteine, was active. The thetins showed slight activity with homocysteine.

Table 3 shows that methionine could also serve as the sole source of methyl and sulfur groups, without limiting the growth rate. Despite marked activity in the presence of sulfate and cysteine, *S*-methyl-L-cysteine as the sole sulfur source severely limited growth. This limitation was alleviated by homocysteine (Table 4), which was not required when methionine was supplied. Whereas cysteine served as a supplementary or limited sulfur source (probably for homocysteine), *S*-methyl-L-cysteine functioned principally as a methyl (or *S*-CH₃) source, even though its cysteine moiety severely limited the rate of growth.

A group of methionine analogues was tested for growth activity (Table 5). The sulfoxide, sulfone, and methyl sulfonium derivatives were inactive, even in the presence of homocysteine. 2-Hydroxy-4-methiol butyric acid was as active as methionine. SAM showed marked activity, and the addition of homocysteine caused additional, although slight, stimulation to 73% growth. Dimethylacetothetin, methyl mercaptan, and α -amino-*n*-butyric acid did not enhance the activity of SAM. The addition of NaI, or adenosine

TABLE 1. Nutritional methionine requirement as a function of glycine and histidine

Medium ^a	Methionine concn		Maximal yield		
	50% Maximal growth	50% Growth inhibition	Amt of protein	Methionine concn	Peak yield age
	mM	mM	mg/ml	mM	hr
Without glycine or histidine	0.32	≥ 0.86	1.76	~ 0.5	96
Without glycine	0.17	0.84	1.96	~ 0.4	120
			0.91	0.86	96
Without histidine	0.33	~ 2	2.36	0.86	78
Complete ^b (glycine and histidine)	0.34	~ 4	2.35	0.86	120
Minimal (OV-40)	0.19	2.1	1.40	0.65	120

^a AV-40 medium with concentrations as follows: L-methionine, 0.86 mM; glycine, 6 mM; L-histidine, 1.3 mM. Of the amino acids (no cysteine) in AV-40, OV-40 medium contains only L-methionine, 1.29 mM; glycine, 9.1 mM; L-arginine, 4.3 mM.

^b When adenosine replaces the glycine requirement, the 50% maximal growth value is 0.27 mM and the calculated 50% growth inhibition value is 0.91 mM, though at 1.7 mM methionine little additional inhibition occurred. This growth value resembles those for media allowing more rapid growth rates ("complete," "without histidine," and "without glycine and histidine") where "glycine" availability is greater whether of exogenous or endogenous origin. The inhibition value is similar only to that found for slow growing glycine-limited cultures on the medium without glycine (+ histidine), suggesting (in contrast to cultures grown without glycine or histidine) that exogenous histidine limits glycine synthesis or metabolic availability (Federation Proc. 21:6, 1962; Bacteriol. Proc., p. 53, 1962).

alone, did not inhibit or stimulate cultures growing with methionine.

Tables 2 through 5 show that active substitutes for methionine must possess the *S*-methyl moiety. Homocysteine, though stimulatory in the presence of *S*-methyl cysteine or SAM, is not required when methionine is present.

Glycyl-L-methionine and L-methionyl-L-methionine also substituted effectively for methionine, at an equal methionine concentration (Table 6). L-methionyl-L-methionine, however, caused lysis of the cultures in the second transfer.

Although, in the presence of glycine and methionine, adenosine (alone or in SAM) did not

affect growth, under other conditions, the availability of the adenosine, as well as the methionine moiety, could be tested. When glycine is omitted from the medium in the presence of histidine, growth retardation can be overcome by adenosine (Daniel et al., Federation Proc. 21:6, 1962).

TABLE 4. *Homocysteine stimulation of S-methyl-L-cysteine-grown cultures*

Compound added ^a	Methionine + cysteine growth ^b	<i>S</i> -methyl-L- cysteine + DL-homo- cysteine growth ^b
	%	%
	(100)	
L-Methionine + L- cysteine	100	
L-Methionine	0	0 (57) ^c
None	6.3 (157) ^c	22 (35) ^d
<i>S</i> -methyl-L-cysteine	5.9 (152) ^c	47 (72) ^d
<i>S</i> -methyl-L-cysteine + L-cysteine	34	100 (63) ^b
<i>S</i> -methyl-L-cysteine + DL-homocys- teine		

^a To AV-40 medium without L-methionine or L-cysteine, compounds added at following concentrations: L-methionine, 0.86 mM; L-cysteine, 2.9 mM; *S*-methyl-L-cysteine, 0.86 mM; and DL-homocysteine, 2.9 mM.

^b All cultures inoculated with 72-hr AV-40 culture to give 0.23 mg of protein per ml. Controls indicated as (100) with maximal growth yields being 2.05 mg protein per ml at 72 hr for methionine + cysteine and 1.29 mg protein per ml for *S*-methyl-L-cysteine + DL-homocysteine at 120 hr. Maximal yield figure is italicized.

^c Calculated as percentage of inoculum level.

^d Calculated as percentage of inoculum level at 7 days.

TABLE 2. *Methyl donors and homocysteine as substitutes for methionine*

Compound added	Concn	Growth ^a	
		Homocysteine present	Homocysteine absent
	mM	%	%
None	—	7	0
L-Methionine	0.86	100	100
<i>S</i> -methyl-L-cysteine	0.86	60	55
Dimethylacetothetin	4.3	17	0
Dimethyl-β-propiothetin	4.3	18	0
Choline	2.9	5	0
Betaine	2.9	9	0
Sarcosine	0.86	13	—
Creatine	0.86	8	0

^a Growth measured as plasmodial protein at 4 days. With methionine, protein content after 4 days was 2.25 mg/ml; without methionine, protein content at 4 days was 0.37 mg/ml. Initial protein content, 0.35 mg/ml; DL-homocysteine concentration, 0.86 mM.

TABLE 3. *Methionine as sole source of sulfur^a*

Medium	Concentration	No. of transfer	Percentage of control	Percentage of <i>S</i> -methyl-L-cysteine control
	mM			
Plus L-methionine	0.86	I-IV	100 (4 days)	
Plus methionine as sole sulfur source	0.86	I	87 (4 days)	
		II-IV	76-93	
Without sulfur	—	I	5 (maximum, 6 days)	
<i>S</i> -methyl-L-cysteine as sole sulfur source	0.86	I	13 (7 days)	23
		II	5	
Plus <i>S</i> -methyl-L-cysteine	0.86	I	56 (7 days)	100
Plus <i>S</i> -methyl-L-cysteine and <i>S</i> -ethyl-L-cysteine	0.43	I	32	57
	1.72	I	13	23

^a Initial plasmodial protein, 0.22 mg/ml; growth on synthetic medium + L-methionine, 2.38 mg/ml at 4 days. Synthetic medium prepared without methionine; additions made as indicated. For testing compound as sole sulfur source, cysteine and inorganic SO₄²⁻ (replaced by Cl⁻) also deleted from medium.

TABLE 5. *Methionine analogues as substitutes for methionine*

Compound added ^a	Age at assay	Protein	Growth (% of control)
	days	mg/ml	
None (inoculum level: 0.38 mg/ml)	6	0.46	3
L-Methionine (control)	4	2.90	100
L-Methionine-DL-sulfoxide	6	0.70	12
L-Methionine sulfone	6	0.84	17
None (inoculum level: 0.39 mg/ml)	6	0.38	0
L-Methionine (control)	3	2.83	100
L-Methionine methyl sulfonium	6	0.52	6
None (inoculum level: 0.44 mg/ml)	4	0.37	—
L-Methionine (control)	3	2.26	100
2-Hydroxy-4-methiol butyric acid ^b	3	2.41	108
None (inoculum level: 0.37 mg/ml)	4	0.39	0.6
L-Methionine (control) ^d	3	2.09	100
S-adenosyl-L-methionine ^c , d	7	1.50	66

^a All tested at 0.86 mM; basic medium was AV-40 minus methionine. Inoculum level expressed as milligrams of protein per milliliter.

^b As calcium salt.

^c As iodide, 85 to 90% pure.

^d Tested at 0.43 mM.

When histidine, not normally required, is omitted in the presence of glycine, adenosine is quite inhibitory (Daniel et al., *Bacteriol. Proc.*, p. 53, 1962); thus, metabolic availability of the adenosine of SAM may be tested in two ways. Table 7 (experiment I, 1.2 mM methionine) demonstrates the stimulation effect of SAM, in the absence of glycine, with histidine present. At a lower methionine concentration (0.32 mM), optimal for this medium (see Table 1), SAM was highly inhibitory owing to the increased available methionine (0.64 mM), though adenosine was also somewhat inhibitory. Increasing the methionine concentration twofold (Table 1, 4 days) gave little additional inhibition. In the presence of glycine without histidine (Table 7, experiment II), adenosine inhibited, but SAM produced only a slight effect. Thus, the adenine of SAM was made available rather slowly, as indicated by growth stimulation (without glycine) or inhibition (without histidine). Since SAM, because of chemical instability (see Discussion), could give rise in the medium to products such as 5'-methylthioadenosine, the growth responses might possibly have been due,

TABLE 6. *Peptides as substitutes for methionine*

Compound added	Transfer	Age at assay	Growth ^a
		days	%
None	I	5	0
	II	6	0
L-Methionine (0.86 mM)	I	4	100
	II	4	100
Glycyl-L-methionine (0.86 mM)	I	5	111
	II	5	78
L-methionyl-L-methionine (0.43 mM)	I	8	98
	II	—	Lysed

^a Growth measured as plasmodial protein. Initial protein density was 0.37 to 0.42 mg/ml; with methionine added, transfer I, 2.08 mg/ml, transfer II, 2.53 mg/ml; without methionine, 0.31 mg/ml.

at least in part, to these, although the low pH for growth would minimize this contribution. The glycine-adenosine-histidine interaction will be described in detail in subsequent publications.

A group of methionine analogues was also tested as methionine antagonists. L-Ethionine and S-ethyl-L-cysteine were the only effective growth inhibitors (Table 8), which supported the observation that the preformed methyl, or S-methyl moiety, of methionine is essential for growth of *P. polycephalum*. S-ethyl cysteine was a better inhibitor in the presence of S-methyl-L-cysteine (Table 3). In contrast, α -methyl-DL-methionine and L-methionine sulfone were relatively ineffective antagonists of methionine and, in the former case, also, of the 2-hydroxy methionine analogue. L-Methionine, itself, was inhibitory at higher levels (Tables 1 and 8). Growth on a semidefined medium (methionine concentration unknown) was less sensitive to ethionine, being unaffected by 0.34 mM. Spherule formation (asexual spore stage), which can occur without exogenous methionine but must rely on an endogenous supply, was, however, quite sensitive, being inhibited 50% by 3.4 μ M DL-ethionine.

DISCUSSION

Of the compounds tested, only three methyl (or S-methyl) donors were effective substitutes for methionine: S-methyl-L-cysteine, 2-hydroxy-4-methiol butyric acid, and S-adenosyl-methionine. From this, it was apparent that *P. polycephalum* could use the methyl or S-methyl group of only a limited number of preformed compounds and could not otherwise transfer or synthesize suitable methyl groups for the synthesis of methionine and other derived methyl-requiring compounds.

TABLE 7. *S*-adenosyl methionine (SAM) as a source of adenosine and "methionine"

Expt no.	Compounds added	Growth (% of control)		
		72 hr	96 hr	144 hr
I ^a	Control (1.2 mM methionine) ^a		30% of 0.32 mM methionine; 18% of control + glycine	(100)
	+ SAM		—	193 ^c . ^d
	+ adenosine		—	170 ^c
	+ glycine		(2.17 mg/ml)	—
	Control (0.32 mM methionine) ^a		(100), 62% of control + glycine	
	+ SAM		40	
	+ adenosine		78	
II ^b	Methionine, 0.86 mM ^b	(100)	(100)	
	+ adenosine	57	90	
	+ SAM	92	(100)	

^a Compounds were added to AV-40 medium without glycine, serine, or threonine: methionine at 1.2 mM or 0.32 mM; SAM or adenosine, 0.32 mM; glycine, 6.0 mM; histidine, 1.3 mM. Inoculum level, 0.21 mg of protein per ml from 72-hr AV-40 culture. All values and calculations corrected for inoculum; 100% value with 0.32 mM methionine, 1.35 mg of protein per ml at 96 hr, and with 1.2 mM methionine, 0.61 mg protein per ml at 144 hr. SAM iodide, 90% pure.

^b Compounds were added to AV-40 medium without methionine, serine, threonine, and histidine (glycine, 6 mM); adenosine or SAM, 0.12 mM. Inoculum level, 0.21 mg of protein per ml from 3-day AV-40 culture. The 100% value at 72 hr was 1.24 mg of protein per ml and at 96 hr, 1.74 mg of protein per ml. Maximal yield figures are italicized. All values and calculations corrected for inoculum.

^c Growth curves for these two cultures develop approximately in parallel, with that for SAM, showing the slightly higher rate as exemplified here.

^d Growth was 54% of control + glycine at 96 hr.

TABLE 8. *Methionine analogues as antagonists*

Compound added ^a	Concn	Growth inhibition
		%
α -Methyl DL-methionine	1.72	3
	8.60	39
	8.60 ^b	17
L-Methionine-methyl sulfonium . . .	1.72	0
	8.60	2
L-Methionine sulfone	1.72	0
	8.60	39
<i>S</i> -ethyl-L-cysteine	1.72	37
	8.60	75
L-Ethionine	1.72	89
	8.60	100
L-Methionine	0.86	0
	4.30	50

^a L-Methionine concentration in all cultures, 0.86 mM. Initial protein density, 0.22 mg/ml; with methionine only, protein content was 2.11 mg/ml. Growth measured at 4 days.

^b Tested with 2-hydroxy-4-methiol butyric acid (0.86 mM) replacing L-methionine: inoculum, 0.24 mg/ml; control (no inhibitor) growth at 4 days, 2.38 mg/ml.

Many of the transmethylnases found in other organisms were lacking (5-7, 26). Significantly, ethionine and *S*-ethyl-cysteine were both good methionine antagonists.

Although *P. polycephalum* was not able to use a number of methyl donors, including methyl mercaptan, the primary defect was apparently its inability to synthesize the methyl or *S*-methyl group, e.g., via the tetrahydrofolic acid pathway (1a, 8, 10), or its inability to make the pathway available for methionine synthesis. Since serine, thymine, folic acid, and cobalamine are not required, such a defect, located in this area, would probably occur after formation of *N*⁵, *N*¹⁰-methylene tetrahydrofolic acid.

Since methionine was the only compound, as a sole sulfur source, found to support a high growth rate, and, since the growth rates obtained with other methionine replacements were substantially stimulated by either cysteine or homocysteine, it appeared that methionine was, in fact, also the main source of these and all sulfur metabolites, except as noted below.

The organism used *S*-methyl-L-cysteine less readily than methionine, but with little or no adaptation. This activity could not be due to

cysteine, present in the medium, but not required (3). Cysteine was presumably derived from L-methionine. However, when S-methyl-L-cysteine was available as the sole sulfur source, sulfate plus cysteine was quite stimulatory. In this case, when the organism was forced to synthesize methionine, cysteine biosynthesis was limiting, very likely as a source of homocysteine, which, although not absolutely required, was stimulatory for S-methyl-cysteine-dependent growth. Apparently, the fundamental limiting factor was the availability of the methyl group, and not of homocysteine nor of other methionine precursors.

S-methyl-L-cysteine also satisfied the methionine requirement of a *Neurospora crassa* mutant (20) and overcame the ethionine inhibition of those yeasts which could be adapted to use this compound as the sole sulfur source (13). The biosynthesis of S-methyl-L-cysteine from methyl mercaptan and serine has been demonstrated by Wolff, Black, and Downey (32). Recently, Newton and Snell (16) have shown a similar reaction catalyzed by tryptophanase.

Direct use of S-adenosyl-L-methionine appears improbable (24, 28, 29). So far, enzymatic conversion of SAM directly to adenosine and methionine has not been reported, although transadenosylation does apparently occur from S-adenosylhomocysteine. SAM synthesis from ATP and methionine, though experimentally reversible in a purified system (14), is not a practical source of methionine. Transmethylation to homocysteine or another four-carbon methionine precursor may occur from this compound, as demonstrated in other systems (22, 27). However, SAM does slowly decompose nonenzymatically to 5'-methylthioadenosine (2, 17), undergoing 24% decomposition in aqueous solution at pH 2.8 after 1 week at 24 C (see reference 24 for summary of chemical characteristics). Under the present culture conditions (pH 4.0 to 4.6, 23 C), the nonenzymatic formation of 5'-methylthioadenosine must be considered as at least a component of the growth activity. However, SAM, when used as a source of adenine, allowed a slightly better growth rate than did adenosine itself (Table 7). This observation suggested that nonenzymatic decomposition products made only a limited contribution to this growth response. In addition, SAM supported similar rates whether acting as a source of methyl groups or of adenine. Norrell and Yall (Bacteriol. Proc., p. 88, 1965) have reported that adenine- C^{14} SAM, used as a growth factor for an adenine-requiring yeast, was recovered completely in nucleic acids, whereas S^{35} -SAM labeled the sulfur amino acids.

Methylthioadenosine is also formed enzymatically from SAM by an *Aerobacter aerogenes* mutant, which can utilize this compound for its methionine requirement, and which incorporates S^{35} and $C^{14}H_3$ labeled 5'-methylthioadenosine into protein methionine (28, 29). The same organism also forms methionine from SAM and homocysteine by direct transmethylation (26). *Candida utilis* appears to incorporate 5'-methylthioadenosine rather directly into SAM (24). Likewise, direct utilization of SAM by *P. polycephalum* could involve transmethylation via 5'-methylthioadenosine as well as directly from SAM. Shapiro and Schlenk (24, 28, 29) have classified and discussed various bacteria on the basis of their ability to utilize SAM and methionine in satisfying growth requirements.

The complete activity of 2-hydroxy-4-methiol butyric acid suggests the ability of the organism to convert the hydroxyl group to an amino group, possibly via an hydroxy acid oxidase (21). This possibility is strengthened by other experiments showing that the organism uses glycolic acid to satisfy its glycine requirement, and that the organism possesses an enzyme converting glycolic acid to glyoxylic acid (Daniel and Babcock, Federation Proc., 25:747, 1966). In addition, both D- and L-methionine are utilized.

The mechanism of growth inhibition by methionine itself is unknown, but it appears to be related to growth rate rather than to lack of specific metabolites, such as glycine, histidine, serine, or adenosine, although the availability of one of these compounds for one-carbon metabolites may regulate the ability to synthesize others (15, 19; F. G. Bull and D. D. Woods, J. Gen. Microbiol. 31:xxiv-xxv, 1963). This is emphasized by a recent indication (18) of an additional site of interaction between purine- and methionine-metabolizing pathways. Of particular interest is the possibility that, since purine synthesis can be limiting for this organism under certain conditions (Daniel and Babcock, *in preparation*; Tables 1 and 7), excess exogenous methionine may limit growth (see Table 1) by trapping adenine, e.g., as SAM (less readily available as adenine). This would agree with the values in Table 1 showing the lowest 50% maximal growth and 50% growth inhibition values for nutritional conditions most limiting to purine synthesis (no glycine, plus histidine). In the absence of histidine, or in the presence of glycine, the inhibition value became larger, while the growth rate was higher. Schmidt et al. (25, see also 7a) have reported large increases in acid-soluble and RNA purines induced by excess methionine feeding of yeasts. The acid-soluble adenine component is known to be mainly

SAM accumulated in large quantities by some yeasts, under these conditions (27).

Methionine is known to repress enzymes of its synthetic and degradative pathways (23), including the N^5, N^{10} -methylene tetrahydrofolate reductase (9), which may not be present in this organism. Clarke (J. Gen. Microbiol. **34**:xi, 1964) has reported that exogenous methionine can inhibit expression of certain reverse adenine auxotroph mutations in a yeast. Excess methionine may also inhibit by allowing methylation, e.g., of glycine, to sarcosine, which is inactive as a source of glycine (Federation Proc. **21**:6, 1962).

Methionine plays a singular role in protein synthesis, at least for bacteria, in that it appears to be the first amino acid in the polypeptide chain (31, see also 1). The significance of methionine as an absolute requirement, in addition to supplying methionine and cysteine for protein and methyl groups, may reside in its role as sole sulfur source. This degradative pattern of sulfur metabolism (11) most likely develops from the inability to synthesize methionine and may limit the synthesis of the other required sulfur metabolites, biotin, and thiamine through a common genetic defect or a series of closely related genetic defects. A possibly similar nutritional relation between biotin and methionine has been described briefly (30) for strains of *Aspergillus* and *Neurospora*.

Attempts to isolate methionine prototrophs from the present strain (4) have been unsuccessful. In such a highly polynucleate organism as *P. polycephalum*, this apparent homogeneity might well indicate the operation of a mutagenic or suppressor metabolism acting to maintain this genome or a stable expression of it. Such a mechanism might, in fact, explain why such an organism exhibits nutritional requirements at all.

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