Repression by Methionine of Cystathionase Formation in *Escherichia coli*

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SUMMARY

Cystathionase catalyses the formation of homocysteine from cystathionine; its formation in cultures of *Escherichia coli* is repressed by the presence of methionine in the growth medium, and to a similar extent to that shown with homocysteine methylase (the enzyme complex which catalyses the conversion homocysteine $\rightarrow$ methionine). Cystathionase, again like homocysteine methylase, is formed rapidly without concomitant growth when repressed organisms are transferred to a medium free from methionine; such enzyme formation is prevented by chloramphenicol, suggesting that *de novo* synthesis of protein is required. The co-repression by methionine of the two enzyme systems fortifies the evidence that cystathionase is a component of the normal pathway of methionine synthesis by *E. coli* and consequently that its substrate, cystathionine, is a normal intermediate. Cystathionase preparations also formed pyruvate from cysteine; this activity paralleled that of cystathionase itself when the methionine status of the medium was changed, and it is concluded that the same protein is responsible for both activities.

INTRODUCTION

In previous work in this laboratory Wijesundera & Woods (1962) showed that L-cystathionine was converted to homocysteine, pyruvate and ammonia by a cystathionase enzyme present in several strains of *Escherichia coli*. Since homocysteine is undoubtedly the immediate precursor of methionine in this organism (see review by Guest & Woods, 1962), these observations supported the view, originating from the properties of methionine auxotrophs of *E. coli* and other micro-organisms, that cystathionine is an earlier intermediate, though there remained some evidence to the contrary (see Discussion). The final reaction by which homocysteine is converted to methionine is a complicated one requiring folic acid derivatives and, in certain circumstances, also cobalamin as cofactors (Guest & Woods, 1962). However, the formation of the enzyme complex (homocysteine methylase), and indeed individual components of it, is repressed by the presence of methionine in the culture medium; the enzymes are, however, rapidly resynthesized when methionine is removed (Rowbury & Woods, 1961; Foster, Rowbury & Woods, 1963). The main objective of the present work was to examine the effect of growth with methionine on the cystathionase content of *E. coli* with conditions under which it could be strictly compared with the known effect on the homocysteine methylase complex.

METHODS

Organisms. The several auxotrophic strains of *Escherichia coli* used were maintained on slopes of tryptic digest of meat agar; stocks were subcultured monthly and stored at about 4° after incubation for 18 hr at 37°. Their growth requirements were: strain PA15 (originally obtained from Dr Barbara Wright) serine or glycine; strain 121/176 (Davis & Mingioli, 1950) methionine or cobalamin; strain 26/18 (obtained from Dr B. D. Davis) homocysteine or methionine.

Organisms for the preparation of washed suspensions or cell-free extracts were grown on the glucose+lactate medium (*GL*) of Guest, Helleiner, Cross & Woods (1960) supplemented with glycine (10 mM), DL-methionine (0.3 mM) and DL-homocysteine (mM) for strains PA15, 121/176 and 26/18, respectively; media were autoclaved for 7 min. at 115°. Growth was in conical flasks (1 l., containing 200–500 ml. medium) which were shaken in air for 16–18 hr at 37° with a circular motion of 220 rotations/min. and radius 2 cm. The size of the inoculum was so adjusted that growth reached the equivalent of 0.4–0.7 mg. dry wt. organism/ml. The cultures were centrifuged at 2200g for 15 min. and the organisms washed once with the culture volume of water. Inocula were derived from cultures (6 ml.) in the same medium seeded from an 18 hr culture on tryptic meat agar and incubated in 1-shaped tubes (150 × 150 × 16 mm.) which were shaken for 8 hr at 37° at 36 oscillations/min. of excursion 10 cm.

Cell-free extracts. Washed organisms were resuspended in water (equiv. 20–30 mg. dry wt./ml.) and subjected to ultrasonic vibration and further treatment as described by Guest *et al.* (1960); in some experiments the dialysed extract was also treated with Dowex-1 resin by the method of these authors. The protein content of the final preparation was determined spectrophotometrically according to Layne (1957).

Extract of heated *Escherichia coli* (strain PA15) was prepared as by Guest *et al.* (1960); it was normally used at 0.1 ml./ml. of reaction mixture.

Cell-free extracts of certain other organisms used in one group of experiments only (Table 3) were made available by other workers in this laboratory.

Study of enzyme formation. Organisms in which enzyme formation had been repressed were obtained by growth in the presence of DL-methionine (10 mM) in medium *GL* supplemented with any other growth factor required by the strain under test. After washing in the culture volume of water, the harvested organisms were suspended (equiv. 3 mg. dry wt./ml.) in the same medium, but without methionine. The suspensions (300–500 ml. in 1 l. conical flasks) were shaken at 37° as described for the original growth of cultures. Samples were taken at intervals (usually 1 hr) for assessment of the extent of growth and for the preparation of cell-free extracts in which enzyme was determined.

Assay of cystathionase, serine hydratase (deaminase) and cysteine desulphhydrase (deaminase). Cell-free extract (equivalent to 1–5 mg. protein) was incubated at 37° for 30 min. in 2 ml. volumes of solution *B* which contained (in 133 mM phosphate buffer, pH 7.4): MgSO₄, 0.25 mM; pyridoxal phosphate, 0.25 mM; L-cystathionine, 10 mM (or DL-allo-cystathionine, 20 mM). The cystathionine was replaced, when required, by D- or L-serine (10 mM) or by L-cysteine (10 mM). The reaction was stopped (and proteins precipitated) by addition of trichloroacetic acid (0.5 ml.;

25 %, w/v). The supernatant fluid after centrifugation was used for the estimation of pyruvate by the method of Friedemann & Haugen (1943), as modified by Wijesundera & Woods (1962).

Methionine synthesis by cell-free extracts. This assay was based on the experiments of Guest *et al.* (1960) and was done by the procedure described by Rowbury & Woods (1961). When required DL-homocysteine (5 mM) was replaced as substrate by L-cystathionine (5 mM) or DL-allocystathionine (10 mM).

Detection and assay of homocysteine. The microbiological method described by Wijesundera & Woods (1962) with *Escherichia coli* strain 26/18 (which responds to homocysteine and methionine) was used, as was the chromatographic procedure used by those authors.

Chemicals. The L-cystathionine used was the batch described by Wijesundera & Woods (1962) isolated from the mycelium of a mutant of *Neurospora crassa*. DL-Allocystathionine was obtained from the California Corporation for Biochemical Research (Los Angeles, U.S.A.). Other special chemicals were as described by Guest *et al.* (1960).

RESULTS

Cystathionase activity of various strains of Escherichia coli

Earlier experiments in this laboratory on the cystathionase of *Escherichia coli* (Wijesundera & Woods, 1962) were made with a pyridoxin auxotroph (B166) and a wild strain (F); furthermore the cell-free extracts then used were prepared by shaking with minute glass balls. It was first necessary to establish that the strain (PA15) chosen for the present work contained the enzyme, and that it survived ultrasonic treatment of the organisms. Cell-free extracts of strain PA15 prepared in this way produced both a keto acid and homocysteine from L-cystathionine; the

Table 1. *The effect of pyridoxal phosphate and magnesium ions on cystathionase activity*

Ultrasonic extract (*Escherichia coli* PA15) was dialysed for 18 hr and one sample treated with Dowex-1 resin. Cystathionase activity was tested in solution B with the stated omissions. Incubation was for 30 min.

Omission	Cystathionase activity (μ mole pyruvate/mg. protein/hr) with extract	
	Dialysed only	Dialysed and treated with Dowex-1
None	0.66	0.71
MgSO ₄	0.70	0.63
Pyridoxal phosphate	0.27	0.08

keto acid was identified as pyruvic acid by the absorption spectrum of its 2,4-dinitrophenyl hydrazone, and the homocysteine both by paper chromatography and by the growth response of *E. coli* strain 26/18. Formation of pyruvate was used thereafter for the routine assay of cystathionase activity in ultrasonically prepared extracts. Pyruvate increased linearly with time up 60 min. and was maximal at a cystathionine concentration of 8 mM; the conditions chosen for normal tests were 10 mM-cystathionine and an incubation period of 30 min.

Cystathionase activity of dialysed extracts was increased about twofold by pyridoxal phosphate; a considerable increase in resolution of the enzyme was obtained by treatment with Dowex-1 resin (Table 1). Contrary to the results of Wijesundera & Woods (1962) the omission of Mg^{2+} had little effect with either the crude or dialysed enzyme preparations (Table 1).

Table 2. *Effect of homocysteine and methionine on the activity of cystathionase*

Cystathionase activity of an extract of *Escherichia coli* PA 15 was assayed in solution B with the stated additions. Incubation was for 30 min.

Addition	Concn. (mm)	Cystathionase activity (μ mole pyruvate/mg. protein/hr)	Inhibition (%)
None	—	1.94	—
DL-Methionine	1.0	1.94	0
	10.0	1.96	0
DL-Homocysteine	0.1	1.84	5
	1.0	1.28	35
	10.0	0.16	92

Table 3. *Cystathionase activity of various strains of Escherichia coli and of other organisms*

The strains of *Escherichia coli* and *Salmonella typhimurium* were grown on basal medium GL and harvested at a similar stage of growth.

Organism	Cystathionase activity (μ mole pyruvate/mg. protein/hr)
<i>E. coli</i> PA 15	1.05
3/62	1.05
121/176	0.93
113/3	0.76
26/18	0.00
<i>Rhodopseudomonas spheroides</i>	0.57
<i>Acetobacter rancens</i>	0.84
<i>Micrococcus denitrificans</i>	0.77
<i>Salmonella typhimurium</i>	0.68
<i>Pseudomonas</i> AM1	0.03
<i>Penicillium</i> Q176	0.03

The breakdown of cystathionine was not affected by the presence of equimolar concentration of DL-methionine, but was inhibited (90 %) by a similar concentration of DL-homocysteine and perceptibly by even one-hundredth of this (Table 2). Wijesundera & Woods (1962) also reported marked inhibition by homocysteine (and also by cysteine and glutathione). It was concluded that the cystathionase enzyme present in *Escherichia coli* strain PA 15 was essentially similar to that studied previously in other strains.

Distribution. The enzyme was absent from ultrasonic extracts of *Escherichia coli* strain 26/18 (Table 3), thus confirming the results obtained by Wijesundera & Woods (1962) who used a different method of disintegration. This strain 26/18 grows with either homocysteine or methionine, but not with cystathionine; a metabolic lesion in the conversion cystathionine $\rightarrow$ homocysteine was therefore expected. The enzyme was present in three other strains of *E. coli*, and in four other organisms, in activity

comparable to that of *E. coli* PA15; with two organisms however (neither of which required methionine for growth) only slight activity was found with the type of preparation tested.

Conversion of cystathionine to methionine. In later work it was desired to compare the effect of growth of the organism in the presence of methionine on the conversion of: (a) cystathionine to homocysteine, (b) homocysteine to methionine, (c) cystathionine to methionine. It was first necessary to establish that (c) occurred by the successive action of the enzymes which catalysed (a) and (b); a direct reductive fission of cystathionine to methionine and glycine was a theoretical possibility, although Wijesundera (1954) found no evidence for this with intact organisms.

Fig. 1. The effect of the concentration of cystathionine on its conversion to methionine by cell-free extracts of *Escherichia coli* PA15.

Table 4. *Conversion of cystathionine to methionine*

Cell-free extracts of the stated strains of *Escherichia coli* were used. The concentration of DL-alloecystathionine was 10 mM; other substrates, 5 mM. Incubation was for 3 hr.

Strain of <i>E. coli</i>	Substrate	L-Methionine formed (μm-mole/mg. protein in 3 hr)
PA15	L-Cystathionine	31
	DL-Alloecystathionine	32
	DL-Homocysteine	70
26/18	L-Cystathionine	1
121/176	L-Cystathionine	0
121/176 + 26/18	L-Cystathionine	12

Methionine was formed by ultrasonic extracts from cystathionine (and allo-cystathionine) at about half the rate at which it was formed from homocysteine (Table 4). Synthesis increased linearly with time up to about 4 hr, and with increasing cystathionine concentration up to about 1.5 mM (Fig. 1); the K_m was

about 0.4 mM. At the lowest concentration of cystathionine tested (0.2 mM) there was about 30 % conversion to methionine.

Serine was required for significant synthesis of methionine from cystathionine (Table 5), as it is also for synthesis from homocysteine (Guest *et al.* 1960). Furthermore, a source of the folic acid cofactor system (either extract of heated organisms or tetrahydropteroylglutamate + cobalamin) was essential (Table 5). Tetrahydropteroylglutamate (in the absence of cobalamin) strongly inhibited the action of the extract of heated *Escherichia coli*. These properties are the same as those described for the conversion of homocysteine to methionine by ultrasonic extracts of the same strain (Guest *et al.* 1960) and would not be expected if cystathionine were cleaved directly to methionine and glycine.

Table 5. *Cofactor requirements for the conversion of cystathionine to methionine*

Methionine synthesis by extracts of *Escherichia coli* PA 15 was tested with L-cystathionine (5 mM) replacing homocysteine and with the other modifications to the reaction mixture indicated. Present, +; absent, -; EHC, extract of heated *E. coli* (see Methods section); H₄PtG, tetrahydropteroylglutamate.

L-Serine	EHC	H ₄ PtG	Cobalamin	L-Methionine formed (μ m-mole/mg. protein in 3 hr)
+	+	-	-	41
-	+	-	-	4
+	-	-	-	0
+	-	+	+	46
+	+	+	-	7
+	-	-	+	0
+	-	+	-	0

It has been shown (Table 3) that extracts of *Escherichia coli* 26/18 lack cystathionase whilst it was known that extracts of strain 121/176 lack homocysteine methylase activity (i.e. ability to convert homocysteine to methionine) unless cobalamin is present (Guest *et al.* 1960; Foster, Tejerina & Woods, 1961). Neither extract alone converted cystathionine to methionine, but a mixture of the two was effective (Table 4), thus confirming the view that methionine formation from cystathionine requires the successive action of cystathionase and homocysteine methylase, and that in this particular case each organism provides the enzyme or enzymes lacking in the other.

Repression of the formation of cystathionase

Although it had already been established that growth in the presence of methionine represses the formation of homocysteine methylase by *Escherichia coli* PA 15 (Rowbury & Woods, 1961), further experiments with this enzyme system were included in the present series with cystathionase so that a strict quantitative comparison might be made. Ultrasonic extracts derived from organisms grown with DL-methionine (10 mM) had only about 20 % of the cystathionase activity of preparations from organisms grown without methionine. Loss of activity increased with increasing concentration of methionine in the growth medium; the sharpest decrease occurred between 0.1 and 1.0 mM, a range over which the effect on homocysteine methylase production was also greatest (Table 6). The degree of repression of enzyme formation at all methionine concentrations was however slightly greater with homocysteine methylase than with cystathionase (Table 6). The overall synthesis of

methionine from cystathionine by ultrasonic extracts of organisms grown with methionine was also greatly decreased compared with the controls, and to an extent similar to that of the decrease in homocysteine methylase activity (Table 7).

Following the terminology of Wijesundera & Woods (1960) organisms and derived enzyme preparations obtained after growth in the presence and absence of methionine will be referred to as 'inactive' and 'active', respectively, the former term now indicating a severely restricted content of both cystathionase and homocysteine methylase.

Table 6. *Effect of methionine on the formation of cystathionase*

The figures in parentheses indicate the percentage decrease in activity due to the presence of methionine during growth. Enzyme assays were on extracts of *Escherichia coli* PA 15 grown with the stated concentration of methionine.

DL-Methionine in growth medium (mM)	Homocysteine methylase (μ m-mole methionine/mg. protein/hr)	Cystathionase (μ mole pyruvate/ mg. protein/hr)
0	60	1.8
0.001	58 (4)	1.6 (10)
0.01	50 (16)	1.5 (17)
0.1	39 (35)	1.4 (22)
1	11 (81)	0.6 (67)
10	8 (86)	0.4 (78)

Table 7. *Effect of growth with methionine on the conversion of cystathionine to methionine*

The figures in parentheses indicate the percentage decrease in methionine formed due to the presence of methionine in the growth medium. Extracts of *Escherichia coli* PA 15 harvested after growth with the stated concentration of methionine were used.

DL-Methionine in growth medium (mM)	L-Methionine formed (μ m-mole/mg. protein in 3 hr) from	
	Homocysteine	Cystathionine
0	83	52
0.001	75 (10)	42 (19)
0.01	71 (15)	36 (31)
0.1	67 (19)	30 (42)
1	21 (75)	15 (71)
10	18 (78)	10 (81)

When 'active' organisms were transferred to a medium containing methionine and incubated, the cystathionase activity of ultrasonic extracts decreased exactly in proportion to the extent of growth. It appears that, as in the case of homocysteine methylase (Rowbury & Woods, 1961), there is no destruction of cystathionase and that it is simply 'diluted out' by new organisms containing only limited amounts of the enzyme.

Effect of homocysteine. Methionine is the ultimate product of the metabolic pathway in which cystathionase presumably functions and it represses the formation of this enzyme. Homocysteine is the immediate product and has been shown above to inhibit the activity of the enzyme; it has however only a small effect on the formation of the enzyme. The presence of DL-homocysteine (10 mM) during growth resulted only in a 20 % decrease in cystathionase activity, an effect which given was by DL-methionine at one-hundredth of the concentration (Table 6).

Relief from repression. When 'inactive' organisms were transferred to a methionine-free medium their cystathionase content (as judged by the activity of ultrasonic extracts) increased rapidly for 3 hr (Fig. 2); the major part of this increase occurred during the first 2 hr, a period during which there was no significant growth. The rate of increase was similar to that of homocysteine methylase, which was assayed in the same cell-free preparations. As expected, the organism also

Fig. 2. Appearance of cystathionase (○) and homocysteine methylase (●) activity in *Escherichia coli* PA 15 following transfer from a methionine-rich to a methionine-free medium. Enzyme activity determined in cell-free extracts of organisms harvested at the stated time.

regained the ability to bring about the over-all conversion (in the presence of serine) of cystathionine to methionine (Fig. 3); the relative rate of synthesis of methionine from cystathionine and homocysteine remained constant during the recovery period.

The increase in cystathionase (and homocysteine methylase) activity on transfer of 'inactive' organisms to methionine-free medium did not occur when chloramphenicol was added (Fig. 4). Since the main increase takes place in advance of growth this effect cannot be a secondary one due to inhibition of growth and provides evidence that relief from repression requires synthesis of new protein; further evidence for this in the case of homocysteine methylase was given by Rowbury & Woods (1961).

Specificity of cystathionase

Allocystathionine. This stereoisomer of the substrate was tested (DL-form) arbitrarily at twice the optimal concentration of L-cystathionine and was degraded at about the same rate (Table 8). The individual isomers of DL-allocystathionine were

Fig. 3. Relative activity of *Escherichia coli* PA 15 in converting homocysteine (O) and cystathionine (●) to methionine following transfer from a methionine-rich to a methionine-free medium. Activity determined in cell-free extracts of organisms harvested at the stated time.

Table 8. *Pyruvate production from certain amino acids by extracts of Escherichia coli*

All substrates except DL-allocystathionine (20 mM) were present at a final concentration of 10 mM.

Strain of <i>E. coli</i>	Substrate	Pyruvate formed (μmole/mg. protein/hr)
PA 15	L-Cystathionine	1.3
	DL-Allocystathionine	1.2
	D-Serine	0.8
	L-Serine	0.1
	DL-Serine	0.8
	L-Cysteine	0.6
	DL-Homocysteine	0.0
26/18	L-Cystathionine	0.0
	L-Cysteine	0.0
	L-Serine	0.1
	D-Serine	0.3

not available for testing, but, unless the enzyme preparations contained a homocysteine racemase, it is certain that D-allocystathionine (which contains a L-homocysteine residue) is the isomer attacked since L-methionine is formed by the further action of homocysteine methylase (Table 4). It cannot, however, be con-

Fig. 4. Formation of cystathionase (○) and homocysteine methylase (●) in the presence (—) and absence (---) of chloramphenicol (0.125 mM). Activity determined in cell-free extracts prepared at intervals after transferring the organisms from methionine-rich to methionine-free medium.

Table 9. Effect of methionine on the formation of D-serine dehydratase (deaminase) and L-cysteine desulphhydrase (deaminase)

Extracts of *Escherichia coli* PA 15 harvested after growth with the stated concentration of methionine were used for the enzyme assay.

DL-Methionine in growth medium (mM)	Pyruvate formed (μmole/mg. protein/hr) from	
	D-Serine	L-Cysteine
0	0.64	0.31
0.001	0.73	0.28
0.01	0.72	0.24
0.1	0.68	0.18
1	0.59	0.05
10	0.59	0.03

cluded that L-allo-cystathionine (containing a D-homocysteine residue) is not attacked since any D-methionine formed would not have been detected by the microbiological assay used. DL-Allo-cystathionine, which, unlike cystathionine, was available commercially, was used in some later experiments.

Cysteine. Crude cell-free extracts formed pyruvate from L-cysteine at about a half the rate from L-cystathionine (Table 8). No attempt was made by purification procedures to determine whether cysteine deaminase activity was due to a distinct enzyme or was a function of the cystathionase enzyme itself. Instead, techniques suggested by the preceding work were used.

Fig. 5. Changes in the activity of the L-cysteine desulphhydrase (●), D-serine dehydratase (○) and cystathionase (⊙) of *Escherichia coli* PA 15 following transfer from methionine-rich to methionine-free medium. Activity, assessed in each case in terms of pyruvate formed, was determined in cell-free extracts of organisms harvested after the time stated.

Ability to metabolize L-cysteine was decreased to 10 % by growth of the organism in the presence of methionine; as with cystathionase the sharpest fall of activity occurred in the range 0.1–1.0 mM-methionine (Table 9). Furthermore, reappearance of cystathionase on transfer of the organisms to methionine-free medium was accompanied by reappearance of cysteine desulphhydrase activity (Fig. 5). *Escherichia coli* strain 26/18, which lacks cystathionase, also lacks cysteine desulphhydrase (Table 8) it is unlikely that a single mutation would affect the production of two proteins. Finally the degradation of cysteine by cell-free preparations of strain PA 15 was inhibited 90 % by DL-homocysteine (10 mM) as was also the case with cystathionine as substrate (Table 2). It is concluded provisionally that cystathionine and cysteine are attacked by the same enzyme.

Serine. Crude cell-free extracts of *Escherichia coli* PA 15 formed pyruvate from

D-serine (though not from L-serine) at two-thirds the rate from cystathionine (Table 8). In this case, however, it is clear from investigations analogous to those used with cysteine that a different enzyme catalyses the reaction. Activity was not significantly decreased by growth in the presence of methionine (Table 9), nor did it increase in parallel with increase of cystathionase (Fig. 5). The strain of *E. coli* (26/18) deficient in cystathionase contained an active D-serine deaminase (Table 8).

DISCUSSION

The growth response of methionine auxotrophs of *Escherichia coli* to possible precursors suggests strongly that cysteine is converted to methionine, as in *Neurospora*, through the successive intermediate formation of cystathionine and homocysteine (Lampen, Roepke & Jones, 1947; Gots & Koh, 1950; Horowitz, 1947). Cystathionine is, however, contra-indicated as an intermediate in *E. coli* by the work of Bolton, Cowie & Sands (1952), who showed that the addition of unlabelled cystathionine to cultures growing with [³⁵S] sulphate as sole source of sulphur did not, as would be expected, decrease the incorporation of ³⁵S into methionine. Wijesundera & Woods (1962) established the presence in the organism of an enzyme which attacked cystathionine (cystathionase) and whose specific products were homocysteine, pyruvate and ammonia; the case for cystathionine as an intermediate was thus strengthened since active enzymic mechanisms (homocysteine methylase) for the further conversion of homocysteine to methionine had been described (see review by Guest & Woods, 1962).

Furthermore, in the present work cell-free extracts of the test strain of *Escherichia coli* catalysed the over-all formation of methionine from cystathionine under conditions, discussed in Results, which make it clear that homocysteine is an intermediate. With intact organisms of the same strain, Wijesundera (1954) found only slight conversion of cystathionine to methionine (10 % of the rate with homocysteine); it is possible that the intact organism has limited permeability to exogenous cystathionine, a possibility which might also explain the results (quoted above) of Bolton *et al.* (1952).

The phenomenon of enzyme repression (in the terminology of Vogel, 1957) provides an interesting and novel approach to the determination of the status of a possible intermediate in a biosynthetic pathway. In several cases the ultimate product is known to repress the formation of enzymes which catalyse a number of steps in the reaction sequence, though not always to the same extent. Examples of this are the biosynthesis of arginine and pyrimidine by *Escherichia coli* and of histidine by *Salmonella typhimurium* (Gorini & Maas, 1958; Yates & Pardee, 1957; Amés & Garry, 1959). One of the earliest described cases of enzyme repression was that of homocysteine methylase, the enzyme complex catalysing the last step of methionine synthesis by *E. coli*; the initial observations of Wijesundera & Woods (1953) and Cohn, Cohen & Monod (1953) were amplified by Wijesundera & Woods (1960) while Rowbury & Woods (1961) showed that relief from repression entailed synthesis of new protein. The present results establish that formation of cystathionase, as well as of homocysteine methylase, is repressed by methionine. It seems reasonable to take this as *a priori* evidence that cystathionase (and therefore cystathionine) is a functional entity in methionine biosynthesis, especially since cystathionase

formation is not markedly repressed by its own immediate product (homocysteine). It is difficult to envisage any explanation other than a control mechanism for this specific effect of methionine. The slight repression caused by homocysteine (at high concentrations) is probably due to methionine formed from it by the growing organism. It may be noted that homocysteine does exhibit the second type of feedback control shown by products, that is, inhibition of the activity of the enzyme.

The extent of repression of cystathionase (and the rate of new enzyme formation on relief from repression) was quantitatively similar to that with homocysteine methylase. Recent studies in this laboratory, reported briefly by Rowbury (1962), show that enzymes which catalyse the synthesis of cystathionine are also repressed, and to a similar degree, by methionine. It is possible therefore that control of methionine synthesis is another example of the 'co-ordinate repression' shown to occur in the histidine pathway by Ames & Garry (1959).

Application of the principles of enzymic repression also permits a novel approach to obtaining evidence, without purifying an enzyme preparation, as to whether two distinct chemical events are or are not manifestations of the activity of the same enzyme. In the present case cysteine desulphydrase (deaminase) activity disappeared and reappeared in parallel with that of cystathionase; this and other evidence led to the conclusion that both activities are a function of the same protein. On the other hand, D-serine dehydratase (deaminase) activity was not affected when cystathionine was repressed or resynthesized; this supports the conclusion of Wijesundera & Woods (1962), based on purification and selective inactivation, that the enzymes which attack D-serine and cystathionine are distinct entities.

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