Fractionation and Kinetic Properties of Rat Liver and Kidney Methionine Adenosyltransferase Isozymes†

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ABSTRACT: Three isozymes of methionine adenosyltransferase (EC 2.5.1.6; MAT-I, -II, and -III) exist in normal rat liver and are conveniently purified (MAT-III to homogeneity) by a three-step column chromatography procedure. MAT-I shows Michaelis–Menten kinetics with a K_m(L-methionine) of 41 μM and a molecular weight of 208 000 and is slightly inhibited by *S*-adenosyl-L-methionine (Adomet). MAT-II, which is also the only isozyme found in normal rat kidney, shows negative cooperativity with a Hill coefficient of 0.7. It has a L-methionine concentration required for half-maximal velocity [$S_{0.5}$(Met)] of 8 μM and a molecular weight of 120 000 and is strongly inhibited by Adomet. MAT-I and -II comprise 15% and 5%, respectively, of total MAT activity in rat liver. The predominant isozyme in rat liver, MAT-III, demonstrates positive cooperativity with a Hill coefficient of 1.8. It has a molecular weight of 97 000 and apparently consists of two subunits of identical molecular weight (47 000). This liver-specific isozyme is strongly activated by both dimethyl sulfoxide and Adomet and has a $S_{0.5}$(Met) of 215 μM.

Methionine adenosyltransferase (MAT)[1] (ATP:L-methionine *S*-adenosyltransferase, EC 2.5.1.6) catalyzes the formation of (−)-*S*-adenosyl-L-methionine according to the reaction

$$\text{L-Met} + \text{adenosine-P}^{\alpha}\text{-P}^{\beta}\text{-P}^{\gamma} \xrightarrow[Mg^{2+}]{K^{+}} \text{(−)Adomet} + \text{P}^{\alpha}\text{P}_i^{\beta} + \text{P}_i^{\gamma}$$

In *Escherichia coli* this has been shown (Markham et al., 1980) to be a sequential mechanism in which random binding of methionine and ATP must occur before the ordered product release of PP_i and P_i, followed by Adomet dissociation. Adomet, thus formed, is involved in numerous biological methylation reactions (Cantoni, 1975), functions as a propylamine donor, after decarboxylation, in the synthesis of spermine and spermidine (Pegg & Williams-Ashman, 1970), can donate a 3-amino-3-carboxypropyl group to uridine in *E. coli* tRNA (Nishimura et al., 1974), serves as an amino group donor in the transamination of 7-oxo-8-aminopelargonic acid (Stoner & Eisenberg, 1975), and is an effector in its own synthesis (Caboche, 1977; Hafner et al., 1977).

MAT from *E. coli* has been purified to homogeneity (Markham et al., 1980) from a recombinant strain derepressed for MAT synthesis which also carried additional *E. coli* MAT genes in a plasmid. This MAT was found to have a molecular weight of 180 000 and consist of four subunits of identical molecular weight (43 000). Two MAT isozymes from yeast have been purified to homogeneity (Chiang & Cantoni, 1977) and found to have similar molecular weights of 110 000. The subunit composition of each isozyme was found to be the same and consisted of two subunits of M_r 55 000 and 60 000. The existence of multiple forms of MAT in yeast has been confirmed by Cherest et al. (1978) and Cherest & Surdin-Kerjan (1981). These researchers studied Adomet-requiring yeast mutants that possessed mutations at two independent gene loci that code for MAT isozymes. The two gene product monomers were found to exhibit Adomet-dependent intra- and inter-dimerization that resulted in the formation of three MAT isozymes.

Mammalian MAT was discovered approximately 30 years ago (Cantoni, 1953), but only recently has a mammalian MAT been purified to homogeneity (Hoffman & Kunz, 1980). The confusion regarding the kinetics of mammalian MAT was partially resolved when the existence of isozymes was recognized (Liau et al., 1977). Three isozymes from rat liver were kinetically defined by varying the assay concentration of Met (Liau et al., 1979) and had K_m(Met) values of 3.6 μM (low K_m), 23 μM (intermediate K_m), and 1.03 mM (high K_m). The low and high K_m isozymes were shown to be functionally associated with methyltransferases, and the sulfhydryl reagent dependence of the high K_m form was noted. Okada et al. (1979) have partially purified two rat liver isozymes, termed α and β, and one rat kidney form, called γ. The α, β, and γ isozymes were found to have molecular weights of 210 000, 160 000, and 190 000 and $S_{0.5}$(Met) values of 17, 500, and 6 μM, respectively. The response of these isozymes to Me_2SO confirmed the original observation of Hoffman & Kunz (1977), and the Hill coefficients, with Met as the variable substrate, suggest the α and γ isozymes are negatively cooperative and the β form positively cooperative (Okada et al., 1981).

A report from this laboratory (Kunz et al., 1980) described a convenient method for separating two MAT isozymes from rat liver, and a preliminary report (Hoffman & Kunz, 1980) extended this to the separation of three rat liver isozymes. We describe in this paper a modified and expedient separation of all three rat liver isozymes and one rat kidney isozyme. The kinetic and molecular properties of these isozymes is explored, as well as their response to the effector Adomet.

Materials and Methods

Materials. L-[^{35}S]Methionine (100–400 Ci/mmol) and Liquifluor were obtained from New England Nuclear. Whatman P81 cellulose phosphate paper disks (2.3 cm) were

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[1] Abbreviations: MAT-I, -II, and -III, isozymes of methionine adenosyltransferase; DTT, dithiothreitol; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Me_2SO, dimethyl sulfoxide; Met, L-methionine; Adomet, (−)-*S*-adenosyl-L-methionine; EDTA, ethylenediaminetetraacetic acid; $S_{0.5}$(Met), defined as the L-methionine concentration required for half-maximal velocity.

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obtained from Reeve Angel. Hepes, dithiothreitol, L-methionine, ATP, and *S*-adenosyl-L-methionine were purchased from Sigma Chemical Co. DEAE-Sephacel, phenyl-Sepharose, and Sephacryl S-300 were products of Pharmacia Fine Chemicals. Dimethyl sulfoxide of spectrophotometric grade was obtained from Matheson Coleman & Bell. All other reagents were of analytical grade.

Female Sprague-Dawley rats (200–300 g) were used as the source of methionine adenosyltransferase.

Buffers. The following buffer systems were used: buffer A, 250 mM sucrose, 10 mM $MgSO_4$, 4 mM DTT, 1 mM EDTA, and 10 mM Hepes, adjusted to pH 7.5; buffer B, 15 mM $MgSO_4$, 4 mM DTT, 2 mM EDTA, 100 mM Hepes, 300 mM KCl, 10 mM ATP, and varying concentrations of L-[^{35}S]methionine, adjusted to pH 7.5 prior to the addition of ATP and methionine; buffer C, 10 mM $MgSO_4$, 4 mM DTT, 1 mM EDTA, and 10 mM Hepes, adjusted to pH 7.5; buffer D, same as buffer C adjusted to 0.3 M KCl; buffer E, 4 mM DTT, 1 mM EDTA, 10 mM Hepes, and 0.3 M KCl, adjusted to pH 7.0; buffer F, same as buffer E without 0.3 M KCl; buffer G, same as buffer F with 40% (v/v) Me_2SO, adjusted to pH 8.0; buffer H, same as buffer C with 150 mM KCl; buffer I, 100 mM ammonium formate adjusted to pH 3.0 with formic acid. Unless otherwise noted, all pH adjustments were made with KOH.

Enzyme Assay. To 50 μL of buffer B was added 20–50 μL of MAT-containing sample (the final volume was adjusted to 100 μL with distilled water). The assays were incubated in a water bath at 37 °C with the time (10–25 min) or enzyme dilution adjusted so that no more than 20% of the L-[^{35}S]-methionine was converted to Adomet. The reactions were stopped by spotting 80 μL of the mixtures on phosphocellulose paper disks and placing these in buffer I (10 mL/disk). The disks were then washed twice more with buffer I followed by an ethanol wash and an ether wash (all at 10 mL/disk). The disks were dried in an oven at 70 °C, and the amount of cationic adenosyl-L-[^{35}S]methionine bound to the disks was determined by counting them under 5 mL of toluene-diluted Liquifluor.

A blank assay, containing water and no enzyme, was carried through the same procedure, and those counts were substracted from the MAT assays. For standardization of the MAT assays, 40 μL of buffer B, which contained varying concentrations of L-[^{35}S]methionine, was spotted on a phosphocellulose disk, dried (without washing), and counted. All assays for kinetic analyses were run in duplicate with less than a 5% difference observed.

Purification of MAT Isozymes. The following is a generalized procedure for separating and purifying MAT isozymes. All operations were carried out at 0–4 °C.

(*1*) *Anion-Exchange Column Chromatography.* Female rats were anesthetized with ether and the livers (or kidneys) rapidly removed, weighed, minced, and homogenized in 4 mL/g of buffer A with a glass homogenizer with a motor-driven Teflon pestle. The homogenate was then centrifuged at 100000*g* for 1 h and the resulting supernatant applied to a DEAE-Sephacel column (3.5 mL bed volume/g of organ) that had been equilibrated with buffer C. The column was then eluted with a linear gradient of buffer C to D (3 times the total bed volume of each buffer) at a flow rate of 15–20 mL h^{-1} cm^{-2}. The fractions collected were assayed for MAT activity, and A_{280} was determined. The high activity fractions were pooled and adjusted to 0.3 M KCl with solid KCl.

(*2*) *Hydrophobic Column Chromatography.* The pooled fractions from above were applied, without concentrating, to a phenyl-Sepharose column (2.5 mL bed volume/g of organ) that had been equilibrated with buffer E. The column was then eluted stepwise with 5 times the column bed volume each of buffers E, F, and G at a flow rate of 25–30 mL h^{-1} cm^{-2}. The fractions collected were assayed for MAT activity, and A_{280} was determined. A peak of MAT activity was associated with each of the buffers (Figure 2), and they were designated MAT-I, MAT-II, and MAT-III by order of elution. The high activity fractions of each isozyme were pooled and concentrated to 5–10 mL by ultrafiltration in an Amicon concentrator equipped with a YM-30 membrane (molecular weight cutoff 30 000).

(*3*) *Gel Filtration Column Chromatography.* Individually concentrated isozymes from above were further purified by passage through a Sephacryl S-300 column (2.5 × 75 cm) that had been equilibrated with buffer H. Fractions were collected at a flow rate of 30 mL/h buffer H and assayed for MAT activity, and A_{280} was recorded. The active fractions were pooled, concentrated to 10 mL by ultrafiltration, divided into 1-mL aliquots, and stored frozen until used for kinetic studies. MAT-III was found to be labile, and therefore, kinetic studies with this form were completed soon after purification (within 24 h).

Molecular Weight Determination. Gel filtration chromatography was carried out at 4 °C on a column of Sephacryl S-300 (1 × 100 cm) eluted with buffer H. The apparent molecular weights of the isozymes were determined by using catalase (M_r 240 000), aldolase (M_r 158 000), bovine serum albumin (M_r 67 000), and ovalbumin (M_r 45 000) as marker proteins. Isozyme purity and subunit structure were assessed by polyacrylamide gel electrophoresis (Davis, 1964) under nondenaturing conditions and in sodium dodecyl sulfate, respectively.

Calculation of Data. Since Eadie–Hofstee plots are more sensitive in detecting homotropic effects in enzymes of low cooperativity (Hensley et al., 1981), they were used for kinetic analyses of the isozymes. The *y* intercept and slope of an Eadie–Hofstee plot for MAT-I (determined by linear regression) were taken directly as the V_{max} and K_m, respectively, for this noncooperative isozyme. Since the Eadie–Hofstee plots of MAT-II and -III were nonlinear, V_{max} was estimated by linear regression to the *y* intercept at high methionine concentration. The value for $^1/_2V_{max}$ was used to determine the $S_{0.5}$(Met) by inspection of the velocity vs. substrate plot. The V_{max} determined above was also used to calculate the Hill coefficients for MAT-II and -III. Hill coefficients (n_H) were obtained from the slopes at $^1/_2V_{max}$ when the data were plotted according to Changeux (1963).

Results

Purification of Rat Liver Isozymes. A typical DEAE-Sephacel column chromatography profile for 50 g of rat liver is shown in Figure 1. The peak of MAT activity eluted in the gradient between 0.21 and 0.24 M KCl and was reasonably well separated from the broad A_{280} peak. This step resulted in an 8-fold purification of total MAT. Fractions 58–69 from above were applied to a phenyl-Sepharose column and eluted stepwise with buffers E, F, and G (Figure 2). Buffer E, which contains 0.3 M KCl, eluted a peak of MAT activity which was designated MAT-I and resulted in a further 1.3-fold purification of this isozyme. MAT-II was eluted by dropping the concentration of KCl to zero (buffer F) and was further purified 2.2-fold by this step. Buffer G, which contains 40% Me_2SO, removed the more hydrophobic MAT-III isozyme from the phenyl-Sepharose column and resulted in a further purification of 19.3-fold for this form. The relative amounts

Table I: Properties of MAT Isozymes

source	K_m or $S_{0.5}$[a] (μM Met)	n_H[b]	M_r[c]	subunits	effect[d] of Me$_2$SO	effect[d] of Adomet
rat liver						
MAT-I (4)[e]	41.0 (±4.0)[f]	1.02 (±0.02)	208 000	ND[g]	0	–
MAT-II (2)	8.3	0.76	120 000	ND	–	– – –
MAT-III (5)	215 (±25)	1.80 (±0.18) 1.00[h]	97 000	2 × 47 000	+ + +	+ +
rat kidney						
peak 1 (3)	8.1 (±0.1)	0.68 (±0.05)	120 000	ND	–	– – –
peak 2 (2)	6.0	0.75	ND	ND	–	– – –

[a] K_m refers to MAT-I and $S_{0.5}$ refers to MAT-II, MAT-III, and the kidney isozymes. [b] n_H is the Hill coefficient obtained with Met as the variable substrate. [c] M_r is the apparent molecular weight from calibrated gel filtration. [d] (0) No effect; (–) inhibition; (+) activation. [e] Numbers in parentheses refer to the number of separate isozyme preparations from rat kidney or liver. [f] (±SEM). [g] ND, not determined. [h] At 500 μM Adomet.

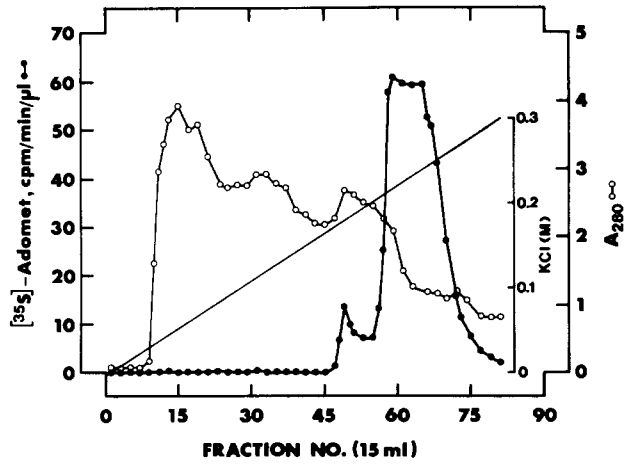

FIGURE 1: DEAE-Sephacel chromatography profile of 50 g of female rat liver 100000*g* supernatant. The column (2.5 × 36 cm) was eluted with a linear gradient of 600 mL of buffer C to 600 mL of buffer D at 60 mL/h. Fractions of 15 mL were collected, and 50-μL aliquots were assayed, for 25 min at 37 °C and 25 μM Met, for MAT activity.

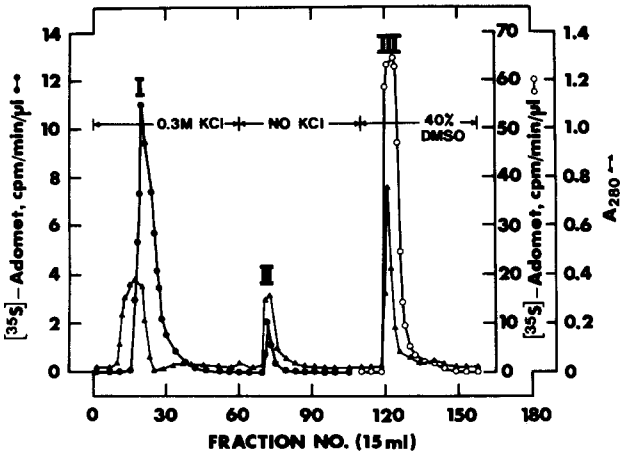

FIGURE 2: Phenyl-Sepharose elution profile of DEAE-Sephacel-purified MAT from rat liver. The rat liver MAT eluted (150 mL) from the DEAE column was applied to a phenyl-Sepharose column (2.5 × 30 cm) and eluted stepwise with 750 mL each of buffers E, F, and G. Fractions of 15 mL were collected at a flow rate of 100 mL/h, and 50-μL aliquots of these fractions were assayed at 37 °C and 25 μM Met for 25 min to determine MAT activity. Each isozyme was purified, after concentration, on a Sephacryl S-300 column.

of MAT-I, -II, and -III eluted from the phenyl-Sepharose column, based on total activity, were found to be 15%, 5%, and 80%, respectively. The isozymes were then individually further purified by gel filtration chromatography on Sephacryl S-300 (not shown). Typical purification of 20-, 150-, and 300-fold were obtained for MAT-I, -II, and -III, respectively. Final specific activities (nanomoles of AdoMet per hour per milligram of protein at 5 mM ATP and 25 μM methionine) were in the range of 75 for MAT-I, 600 for MAT-II, and 1200 for MAT-III. A highly detailed description of the method and results of purification is found in Hoffman (1983).

Purification of Rat Kidney Isozyme. The peak of MAT activity from rat kidney was eluted at approximately the same position (0.21–0.23 M KCl) as liver MAT by the linear KCl gradient on the DEAE-Sephacel column (not shown). A typical phenyl-Sepharose elution profile, following anion-exchange chromatography, for 8.2 g of rat kidney is shown in Figure 3. No peak of MAT activity corresponding to liver MAT-I was eluted from the phenyl-Sepharose column. Two peaks of MAT activity (peak 1 and peak 2 in Figure 3), corresponding in elution positions to liver MAT-II and -III, were observed. These two peaks, however, show the same kinetics (see below) as liver MAT-II and are assumed to be the same isozyme. These two peaks of MAT activity were additionally purified by gel filtration prior to the following kinetic analyses, yielding specific activities comparable to that of liver MAT-II.

Kinetic Properties of MAT Isozymes with Respect to Methionine. The following study of kinetic properties was

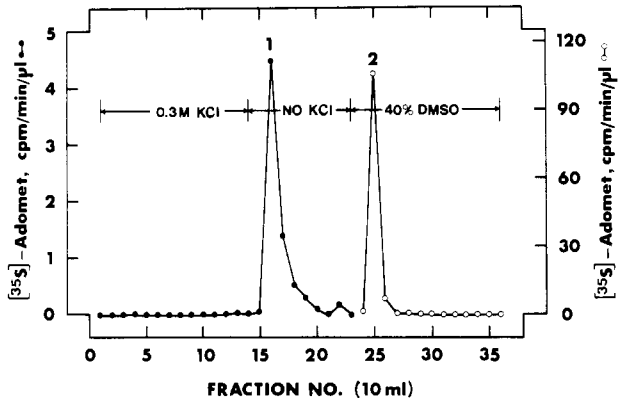

FIGURE 3: Phenyl-Sepharose profile of DEAE-Sephacel-purified MAT from 8.2 g of rat kidney. DEAE-eluted rat kidney MAT (40 mL) was applied to a phenyl-Sepharose column (1 × 25 cm) and was eluted with 100 mL each of buffers E, F, and G. Fractions of 10 mL were collected at a flow rate of 50 mL/h, and 50-μL aliquots were assayed at 37 °C and 25 μM Met for 20 min to determine MAT activity. Peaks 1 and 2 were purified, after concentration, on a gel filtration column.

directed toward more physiological conditions of pH, ionic strength, and ATP concentrations, and thus these properties have not been varied. Our unpublished data show pH vs. activity profiles to be relatively flat between pH 7.5 and 9, with the maximum at 9 being 15–20% higher than at 7.5.

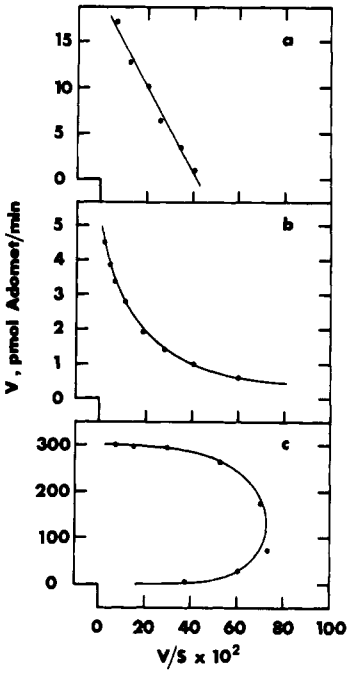

FIGURE 4: Eadie–Hofstee plots of purified rat liver isozymes. Each isozyme was purified by the three-column procedure described under Materials and Methods and assayed at variable methionine concentrations. MAT-I (a) gives a straight line indicative of noncooperative kinetics, MAT-II (b) shows negative cooperativity, and MAT-III (c) demonstrates positive cooperativity. S is given in μM Met. Reaction mixtures of 100 μL contained 20 μg of MAT-I, 1 μg of MAT-II, or 15 μg of MAT-III.

(*1*) *From Rat Liver.* MAT-I was found to conform to Michaelis–Menten kinetics and gave a straight line on an Eadie–Hofstee plot (Figure 4a). MAT-I was found to have an intermediate K_m(Met) value of 41.0 μM (±4.0 μM), as the concentration of Met was varied between 1 and 250 (Table I). A Hill plot of the data gave a Hill coefficient (n_H) of 1.02 (±0.02) which also indicates a noncooperative isozyme (data not shown).

The Eadie–Hofstee plot for purified MAT-II (Figure 4b) gave a concave upward curve over the concentration range 0.1–100 μM Met. V_{max}, $S_{0.5}$(Met), and n_H were calculated as described under Materials and Methods. MAT-II was found to have an $S_{0.5}$(Met) of 8.3 μM and an n_H of 0.76. The Eadie–Hofstee curve and n_H value indicate that this is a negatively cooperative isozyme.

MAT-III from rat liver also deviated from linearity on an Eadie–Hofstee plot (Figure 4c). For this isozyme a concave downward curve was seen over the concentration range 0.025–4 mM L-Met. $S_{0.5}$(Met), calculated as for MAT-II, was 215 μM (±25 μM) with an n_H of 1.80 (±0.18). This isozyme, like MAT-II, shows homotropic modulation but in this case is positively cooperative. We have not reexamined the three liver MATs with regard to ATP as the variable substrate. However, our previous work indicated that the high K_m, Me_2SO-activated form we now call MAT-III was positively cooperative with respect to ATP (Kunz et al., 1980).

(*2*) *From Rat Kidney.* Phenyl-Sepharose chromatography of DEAE-Sephacel-purified kidney MAT gave two peaks of MAT activity (Figure 3). When peak 1 was assayed over the concentration range 0.1–100 μM Met and the data recorded as an Eadie–Hofstee plot, a concave upward curve, similar to that of liver MAT-II, was observed. The $S_{0.5}$(Met) for this peak was calculated to be 8.1 μM (±0.1 μM) and the n_H = 0.68 (±0.05). Similarly, kidney peak 2 from the phenyl-Sepharose column exhibited a concave upward curve, had an

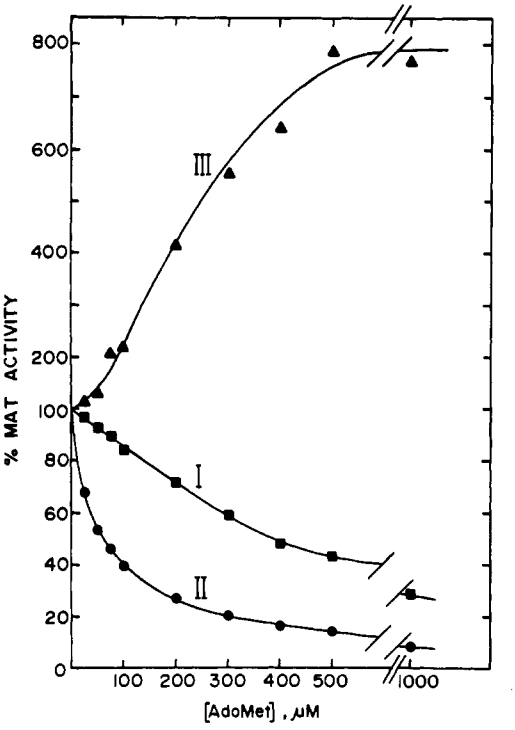

FIGURE 5: MAT isozyme activity as a function of Adomet concentration. The liver isozymes were assayed at 25 μM Met and 5 mM ATP in the presence of variable concentrations of Adomet with MAT concentrations as in Figure 1.

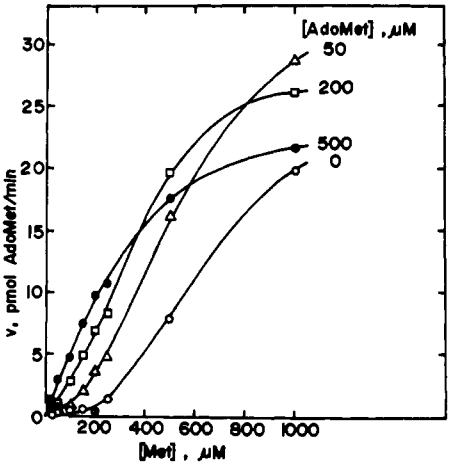

FIGURE 6: Effects of Adomet on liver MAT-III. MAT-III was purified and assayed as described by using 5 mM ATP and variable initial concentrations of Met and Adomet as shown, with 1.5 μg MAT in each 100 μL of reaction mixture.

$S_{0.5}$(Met) of 6.0 μM, and had an n_H of 0.75 (Table I). Kinetically, liver MAT-II and the two peaks of MAT activity from kidney appear to be the same negatively cooperative isozyme. We as yet have no explanation for the fractionation of kidney MAT into two peaks on phenyl-Sepharose.

Effects of Adomet. The effects of increasing Adomet concentrations on MAT activity of the three liver isozymes at a fixed concentration of L-Met (25 μM) are shown in Figure 5. The activity of MAT-I and -II was decreased by 50% at Adomet concentrations of 400 and 60 μM, respectively. MAT-III, on the other hand, was markedly activated by Adomet with a maximum 8-fold stimulation at 500 μM. The activity of the two kidney MAT peaks was inhibited to the same extent as liver MAT-II (Table I).

A more complete study of the effects of Adomet on MAT-III is shown in Figure 6. As the Adomet concentration

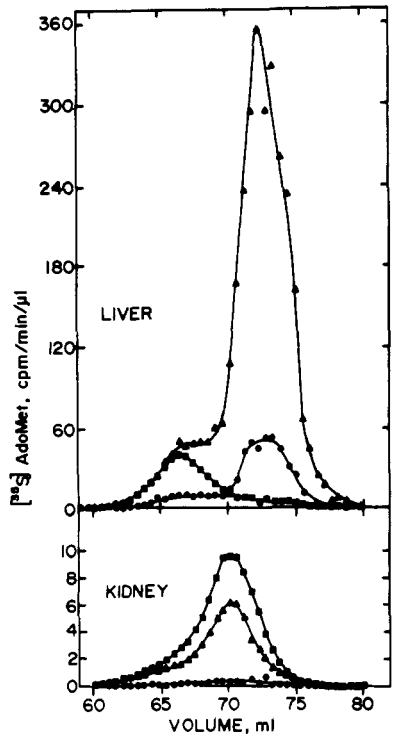

FIGURE 7: Comparison of the effects of Adomet and Me_2SO on MATs fractionated by gel filtration. Postribosomal supernatants were prepared, and 0.5-mL portions corresponding to 125 mg of liver or kidney were chromatographed on a 1.2 × 100 cm column of Sephacryl S-300 eluted with buffer at 15 mL/h. Fractions of approximately 0.5 mL were collected in tared containers and assayed for volume by weighing, and for MAT by using 5 mM ATP and 25 μM Met. (■) Control; (▲) 10% Me_2SO; (●) 1 mM Adomet.

is increased to 500 μM over a L-Met concentration range of 0.01–1 mM, the sigmoidal nature of the velocity vs. substrate plot is abolished. The n_H value decreases from 1.8 to 1.0 (Table I) in the absence of Adomet and in the presence of 500 μM Adomet, respectively. This suggests a shift from positive cooperativity to noncooperativity at the increased Adomet level.

The effects of Adomet on the various MATs can be combined with fractionation to diagnose the types of isozymes present as demonstrated in Figure 7. Fractionation of liver or kidney extracts by gel filtration followed by MAT assays with no additions, with 10% Me_2SO, or with 1 mM Adomet gives distinctive elution profiles. Adomet is even more effective than Me_2SO in distinguishing the three MAT types, since it gives moderate inhibition of I (highest molecular weight), complete inhibition of II (intermediate molecular weight; see kidney profile), and activation of III (lowest molecular weight). It is also evident from this figure that gel filtration will not permit the fractionation and detection of all three forms. In particular, the small amounts (5% of total) of MAT-II in normal adult liver will be masked by the greater amounts of I and III eluting on either side. This is the reason Okada et al. (1981) reported only two forms in adult rat liver. Hydrophobic chromatography should be the method of choice for quantitative measurements of MAT mixtures.

Molecular Properties. On the basis of gel filtration on Sephacryl S-300 (data not shown) the apparent molecular weights of the three liver isozymes were found to be the following: MAT-I, 208 000; MAT-II, 120 000; MAT-III, 97 000. The purification procedure employed in this study resulted in homogeneous MAT-III, which was found to consist of two identical subunits of M_r 47 000. Polyacrylamide gels, under nondenaturing conditions, showed four to six bands for MAT-I and MAT-II purified as above.

Discussion

The results of these investigations indicate that normal rat liver contains three kinetically and molecularly distinct isozymes of MAT (Table I) and not two, as suggested by Okada et al. (1981). These can be conveniently separated and purified (MAT-III to homogeneity) by a three-column chromatography procedure. MAT-I is similar to *E. coli* MAT (Markham et al., 1980) in that it shows Michaelis–Menten kinetics and has an apparent molecular weight of approximately 200 000. This isozyme has a K_m(Met) of 41 μM and an n_H of 1.02, shows little or no stimulation by 10% Me_2SO, and is slightly inhibited by Adomet. It does not show the negative cooperativity observed for its α counterpart by Okada et al. (1981). (This may be due to contamination of their α-isozyme preparation by MAT-II.) MAT-II from liver shows the negative cooperativity (n_H = 0.76) observed for the two yeast isozymes (Chiang & Cantoni, 1977) and has a similar apparent molecular weight (120 000 for liver MAT-II and 110 000 for the yeast isozymes). It has an $S_{0.5}$(Met) of 8.3 μM and is strongly inhibited by Adomet.

MAT activity from the kidney appears to be limited to one isozyme type, and this form is kinetically indistinguishable from liver MAT-II (Table I). The γ isozyme isolated from rat kidney by Okada et al. (1981), except for a discrepancy in molecular weight, is similar to our MAT-II.

The liver-specific, "high K_m" MAT-III isozyme is different than both the *E. coli* and yeast enzymes in that it shows positive cooperativity (n_H of 1.8). In common with the yeast isozymes is the Adomet-stimulated MAT activity of this form. MAT-III is also strongly activated by Me_2SO, has a $S_{0.5}$(Met) of 215 μM, and in a homogeneous preparation has a molecular weight of 97 000.

The positively cooperative modulation of MAT-III by L-Met and Adomet occurs at physiological metabolite concentrations; that is, at 50–150 μM Met, 50–200 μM Adomet shows strong activation (Figure 6). Thus an increase in Met in the liver would increase Adomet and further stimulate the rate of Adomet synthesis, permitting the rapid clearance of excess Met. Such a mechanism may be necessary to prevent the toxic effects caused by the diversion of excess Met into transamination and oxidation (Mitchell & Benevenga, 1978). In tissues such as kidney which have only MAT-II-type activity (Hoffman & Kunz, 1980), normal Met levels of 50–100 μM are an order of magnitude higher than the $S_{0.5}$(Met) (6–8 μM). This means that the velocity of Adomet synthesis will be near maximal and relatively unaffected by fluctuations (particularly increases) in Met. Since MAT-II is very sensitive to Adomet inhibition, the steady-state levels of Adomet (dependent mainly on the rate of utilization) will be the primary determinant of the rate of Adomet synthesis.

A low K_m isozyme similar to MAT-II has been found to persist or increase, while other isozymes decrease, in rat hepatomas induced by *N*-(2-fluorenyl)acetamide (Tsukada & Okada, 1980), and the existence of this γ-like isozyme in rat hepatomas has been likened to the fetal liver condition (Okada et al., 1980). An altered intermediate K_m isozyme, similar to MAT-I, has been found in rat Novikoff hepatomas and in various human malignant tumors (Liau et al., 1980). The link here between MAT isozymes and malignancy suggests that isozyme-specific inhibitors could be of potential chemotherapeutic value. Studies on the specific inhibition of MAT isozymes by such methionine analogues have been reported (Hoffman & Sullivan, 1982), with the interesting finding that MAT-III is usually activated by analogues which inhibit the

other two forms.

Registry No. MAT, 9012-52-6; Met, 63-68-3; Adomet, 22365-11-3; Me_2SO, 67-68-5.

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Kinetic Study of the Interaction between Ribulosebisphosphate Carboxylase/Oxygenase and Inorganic Fluoride†

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ABSTRACT: The effect of inorganic fluoride on the reactions catalyzed by ribulosebisphosphate carboxylase/oxygenase has been characterized with the fully activated enzyme. Fluoride inhibits both reactions, and the concentration required to inhibit the activity of the magnesium-activated enzyme 50% is 2 mM when reactions are carried out at pH 8.3. Inhibition is strongly pH dependent with an apparent pK_a of 8.8. The inhibition kinetics were studied. It was found that inhibition is mixed but close to noncompetitive with respect to CO_2 and uncompetitive with respect to ribulose 1,5-bisphosphate. The mechanism of interaction between fluoride and the enzyme is discussed, and a model is proposed in which fluoride interferes with the reactions by displacing a catalytically important ligand, probably a water molecule, from the activator metal.

Ribulose-1,5-biphosphate carboxylase/oxygenase catalyzes the two reactions (Calvin & Massini, 1952; Andrews et al., 1973)

$$\text{ribulose bisphosphate} \xrightarrow{CO_2} \text{2,3-phosphoglycerate} \quad (1)$$

$$\text{ribulose bisphosphate} \xrightarrow{O_2} \text{3-phosphoglycerate} + \text{2-phosphoglycolate} \quad (2)$$

Reaction 1 is the primary step of CO_2 fixation in C_3 photosynthesis, and reaction 2 is believed to be the initial reaction of photorespiration (Lorimer, 1981). The substrates CO_2 and O_2 are competitive with each other with the consequence that O_2 inhibits CO_2 fixation in photosynthetic organisms (Badger & Andrews, 1974).

Both reactions have an absolute requirement for a divalent metal ion. Mg^{2+} is the best activator, but Mn^{2+}, Co^{2+}, Ni^{2+}, and Fe^{2+} also support activity (Weissbach et al., 1956; Christeller, 1981).

Metal activation has been shown to occur by the slow reversible addition of CO_2 and a metal ion to the enzyme to form an enzyme–CO_2–M^{2+} complex, which is the catalytically active species (Lorimer et al., 1976). The CO_2 molecule in this activation reaction is bound as a carbamate (Lorimer & Miziorko, 1980) and does not react with ribulose 1,5-bis-

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