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PURIFICATION AND CHARACTERIZATION OF RAT HEART AND BRAIN CATECHOL METHYLTRANSFERASE

RONALD T. BORCHARDT and CHAO FU CHENG

Department of Biochemistry, McCollum Laboratories, University of Kansas, Lawrence, Kan. 66044 (U.S.A.)

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Summary

In an effort to detect the similarities and differences in the properties of rat heart, brain and liver catechol methyltransferase (*S*-adenosyl-L-methionine: catechol *O*-methyltransferase, EC 2.1.1.6), we have determined the cellular distribution of this enzyme activity and extensively purified the soluble and microsomal enzymes present in these tissues. Purification of soluble heart (688-fold) and brain enzymes (240-fold) were achieved using an affinity chromatographic system. The properties of these enzymes were compared with respect to their molecular weights, substrate specificities, inhibitor specificities and immunological properties. The characteristics of the enzyme active sites were investigated using various methyl acceptor substrates and various analogs of *S*-adenosylmethionine as methyl donors. A series of analogs of *S*-adenosylhomocysteine was also evaluated as inhibitors of these enzymes. The immunological properties of the purified soluble and microsomal enzymes from heart and brain were investigated using an antibody isolated from rabbits which had been immunized with the soluble rat liver enzyme. In general the properties of catechol methyltransferases isolated from heart and brain were similar to the properties of the enzyme isolated from liver. Some minor differences in substrate and inhibitor specificities were observed which might suggest slight differences in the active sites of these enzymes.

Introduction

Catechol methyltransferase (*S*-adenosyl-L-methionine:catechol-*O*-methyltransferase, EC 2.1.1.6) catalyzes the transfer of a methyl group from *S*-adenosylmethionine to a catechol substrate resulting in the formation of the *meta* and *para*-O-methylated products [1,2]. This enzyme, which is widely distributed in mammalian tissues, plays a primary role in the extraneuronal inactivation of endogenous catecholamines and the detoxification of xenobiotic

catechols [2]. The highest level of this O-methylation activity is generally found in liver, consequently the enzymes isolated from rat [1—20] and human liver [12,21,22] have been most extensively studied. Less attention has been directed to catechol-methyltransferase in tissues other than liver, although some properties of the enzymes in rat and human brain [12], human placenta [23], human erythrocytes [24—26] and lung [27] have been reported.

Since our laboratory has been interested in the design of inhibitors of catechol-methyltransferase [16—20, 28—34], we undertook a study in an effort to detect differences in the properties of this enzyme in various rat tissues. In this way, we would be in a position to take advantage of these differences for the design of tissue specific inhibitors of this enzymatic activity. Therefore, we report here the isolation and purification of catechol-methyltransferase from rat heart and brain, and a comparison of the properties (substrate specificities, inhibitor specificities, molecular weights, immunochemical properties, etc.) of these enzymes with the properties of the purified rat liver enzyme.

Materials and Methods

Chemicals

S-Adenosyl[Me-^{14}C]methionine (New England Nuclear, 55.0 mCi/mmol) was diluted to 10 μCi/ml and stored at −21°F. *S*-Adenosylmethionine chloride (Sigma) was stored as a 0.01 M aqueous stock solution. The following compounds were obtained from the indicated sources: 3,4-dihydroxybenzoate (Aldrich); bovine serum albumin (Calbiochem); lysozyme (Chemical Procurement Laboratories); *S*-adenosyl-L-homocysteine, calcium phosphate gel, L-epinephrine, L-norepinephrine hydrochloride, ovalbumin, pepsin, Sephadex G-25, Sephadex G-100, Triton X-100, trypsin (Sigma). The following compounds were synthesized using published procedures: 3,4-dihydroxyacetophenone [35]; *S*-3′-deoxyadenosyl-L-[*Me*-^{14}C]methionine, *S*-tubercidinyl-L-[*Me*-^{14}C]methionine, *S*,N^6-methyladenosyl-L-[*Me*-^{14}C]methionine, *S*-3-deazaadenosyl-L-[*Me*-^{14}C]methionine, *S*-8-asaadenosyl-L-[*Me*-^{14}C]methionine [34]; *S*-tubercidinyl-L-homocysteine and the other analogs of *S*-adenosyl-L-homocysteine listed in Table IV [28—32]. The 3,4-dimethoxy-5-hydroxyphenylethylamine-agarose conjugate used in the affinity chromatographic purification of rat heart and brain catechol methyltransferase was synthesized using a previously published procedure [15]. Protein concentrations were determined using the method of Lowry et al. [36].

Catechol methyltransferase assay

The soluble enzyme activities were determined using *S*-adenosyl-[*Me*-^{14}C]-methionine and 3,4-dihydroxybenzoate as substrates according to a previously described radiochemical assay [11,18]. The enzyme activties present in the particulate fractions (nuclear, mitochondrial or microsomal) were determined using a similar assay [11,18] except dithiothreitol was not included in the incubation mixture, because of the presence of a thiol-*S*-methyltransferase in these fractions [11 and Borchardt, R.T. and Cheng, C.F., unpublished observations]. The kinetic constants (K_m and V) and the inhibition constants (K_I) were calculated according to the methods of Cleland [37] using a Hewlett-Packard 2100A digital computer.

Enzyme subcellular distribution studies

The subcellular fractionation of rat liver was carried out using previously described procedures [5,11,15]. Rat brain was homogenized in 10 vols. (v/w) of ice-cold 1 mM phosphate buffer, pH 6.7 (0.32 M sucrose, 1 mM $MgCl_2$), whereas rat heart was homogenized in 5 vols. (v/w) of 5 mM phosphate buffer, pH 7.4 (0.32 M sucrose). Subcellular fractionation was achieved using techniques similar to that described previously for rat liver [5,11,15]. Brain synaptosomes were purified from the mitochondrial fraction using a two-step discontinuous Ficoll-sucrose gradient [39,40] consisting of 20-ml layers of 14% (w/v) and 7.5% (w/v) Ficoll in 0.32 M sucrose. The gradient was centrifuged at 25 000 rev./min (Beckman SW 25.2 rotor) for 45 min and the synaptosomal fraction was obtained from the interface of the 7.5—14% Ficoll sucrose layers.

Enzyme purification

The soluble brain and heart catechol methyltransferases were purified using techniques similar to those described earlier for the purification of the rat liver enzyme [5,15]. The enzyme activity present in the microsomal fractions of rat heart and brain were purified using techniques similar to those described earlier for the purification of the rat liver microsomal catechol methyltransferase [11]. The microsomes were purified by a two-step discontinuous sucrose gradient followed by solubilization of the heart and brain microsomal enzymes with Triton X-100.

Molecular weight determinations by gel filtration

Molecular weight estimations were performed at 4°C using a Sephadex G-100 column (1 × 58 cm) prevously equilibrated with 10 mM phosphate buffer, pH 7.0 (1.0 mM dithiothreitol). The column's void volume was determined using Blue Dextran 2000. Freshly prepared high speed supernatants and purified soluble enzyme after calcium phosphate gel adsorption from liver, brain and heart were used in this study. A sample (0.5 ml) of the enzyme preparation was applied to the Sephadex G-100 column and the column eluted at a flow rate of 6.6 ml/h collecting 1.0-ml fractions. Each fraction was assayed for enzyme activity using the procedures described above. Estimations of molecular weights were made from a calibration curve of molecular weights vs. elution volumes for standard proteins (e.g. lysozyme, 14 300; trypsin, 23 000; pepsin, 35 000; ovalbumin, 45 000; and bovine serum albumin, 68 000).

Immunochemical studies

The anti-liver catechol-methyltransferase γ-globulin used in these studies was obtained from New Zealand rabbits immunized with purified liver catechol-methyltransferase. The immunization schedule and the purification of the anti-liver catechol-methyltransferase γ-globulin have been previously described [40,41]. Immunodiffusion experiments were performed on 1.5% agarose (Noble Difco) in phosphate buffered saline, pH 7.2. Immunodiffusion plates were incubated in a moisture chamber at 4°C for 24 h. After washing with cold distilled water, the plates were stained with 1% (w/v) Amido Black solution in 20% acetic acid/50% ethanol at room temperature for 1—2 h. Excess dye was

removed by extensive washing with a 10% methanol/20% acetic acid solution and water. To determine the ability of the anti-liver catechol-methyltransferase γ-globulin to inhibit the soluble and microsomal enzymes isolated from rat heart and brain, a constant amount of the enzyme was added to a series of tubes containing increasing amounts of the antibody in 0.1 M phosphate buffer, pH 7.8. The tubes were preincubated at 25°C for 30 min after which residual enzyme activity was determined.

Results

Subcellular distribution of catechol methyltransferase

Examination of the subcellular distribution of catechol-methyltransferase showed that the majority of the enzyme activity appeared in the soluble fractions (liver, 94.8%; brain, 81.3%; heart, 95.1%) with lesser amounts appearing in the nuclear fractions (liver, 0.8%; brain, 4.6%; heart, 1.8%), the mitochondrial fractions (liver, 1.4%; brain, 8.8%, heart, 1.3%) and the microsomal fractions (liver, 3.0%; brain, 5.2%; heart, 1.8%). Rat brain synaptosomes prepared on a discontinuous Ficoll gradient exhibited only 1.3% of the total enzyme activity.

Enzyme isolation and purification

The steps involved in the purification of the heart and brain soluble enzymes were modeled after procedures previously described by Nikodejevic et al. [5] and Borchardt et al. [15] for the purification of liver soluble catechol-methyltransferase. The steps involve differential centrifugation, ammonium sulfate fractionation, Sephadex G-25 chromatography and negative calcium phosphate adsorption. Further purification of these enzymes was achieved by chromatography of the calcium phosphate purified enzymes on an affinity chromatographic system consisting of a 3,4-dimethoxy-5-hydroxyphenylethylamine-agarose conjugate eluting with buffers of increasing ionic strength [15]. Use of these purification techniques resulted in a 680-fold purification of the heart enzyme (Table I), whereas the brain enzyme was similarly purified 240-fold.

In addition to purifying the soluble enzymes present in rat heart and brain, we also have studied the microsomal enzymes present in these tissues. Earlier our laboratory [11] has reported the solubilization and partial purification of rat liver microsomal catechol methyltransferase. In this study we have partially purified the enzyme activities in microsomes obtained from rat brain and heart using a two-step discontinuous sucrose gradient [11]. The purified microsomal fragments having catechol-methyltransferase activity were then solubilized using Triton X-100. Complete solubilization of the heart microsomal enzyme activity was achieved by incubating at 4°C for 30 min with 1% Triton X-100. In contrast the brain microsomal enzyme could be solubilized using 0.6% Triton X-100 under the same incubation conditions.

Molecular weight determinations

Estimations of the molecular weights of the rat liver, heart and brain soluble enzymes were made by determination of the elution volumes on a calibrated Sephadex G-100 column. In Fig. 1 is shown a typical elution pattern when an

TABLE I

PURIFICATION OF RAT HEART CATECHOL METHYLTRANSFERASE

Fraction *	Total ** protein (mg)	Specific activity *** (nmol/mg protein/min)	Purification	Recovery (%)
Homogenate	15 590	0.04	1	—
100 000 X *g* supernatant	3 353	0.13	3.2	69
$(NH_4)_2SO_4$ fractionation	668	0.36	9.1	39
Sephadex G-25 chromatography	413	0.68	16.9	45
$CaHPO_4$ adsorption	146	1.23	30.8	29
Affinity † chromatography	0.78	27.52	688	33

* Fractionation procedures are described in detail in Materials and Methods and ref. 15.

** Protein concentrations were determined by the method of Lowry et al. [36].

*** Assay conditions: 3,4-dihydroxybenzoate concentration, 2.0 mM; *S*-[*Me*-^{14}C]adenosyl-L-methionine (0.05 μCi), concentration 1.0 mM; Mg^{2+}, concentration 1.2 mM; phosphate buffer, pH 7.60. Specific activity expressed as nmol of methylated product formed per mg of protein per min.

† The $CaHPO_4$-purified enzyme (66.44 mg) was chromatographed on a 3,4-dimethoxy-5-hydroxyphenylethylamine-agarose conjugate (1 X 20 cm) eluting with phosphate buffer, pH 7.2 ([phosphate] = 5—60 mM, [EDTA] = 0.2 mM, [Mg^{2+}] = 0.2 mM). See Fig. 1 for typical elution pattern.

aliquot of the high speed supernatant from homogenized rat heart was chromatographed on a Sephadex G-100 column eluting with 10 mM phosphate buffer, pH 7.0. When the heart soluble enzymes at various stages of purification were chromatographed in this same manner only a single peak of activity was

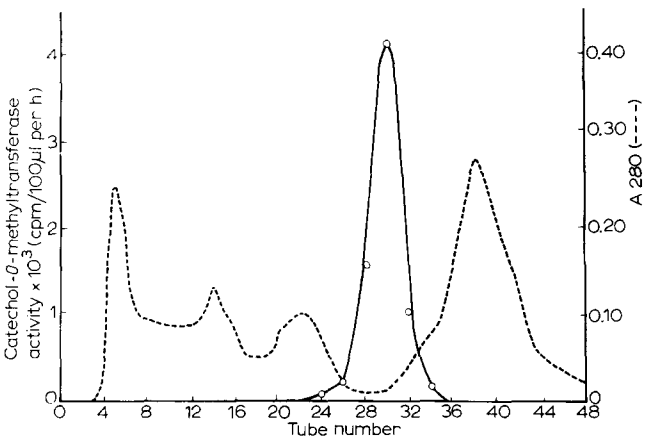


Fig. 1. Elution pattern of rat heart catechol-methyltransferase from Sephadex G-100. A 0.5 ml sample of the 100 000 X *G* supernatant from homogenized rat heart was applied to a Sephadex G-100 column (1 X 58 cm) and eluted with 0.01 M phosphate buffer, pH 7.0 (dithiothreitol, 1.0 mM). The protein concentration (— — —) was estimated by monitoring the eluent at 280 nm. Fractions of 1.0 ml were collected and assayed for enzyme activity (o———o) using the conditions described under Materials and Methods.

observed with an estimated molecular weight of 23 000. Identical results were obtained using the soluble enzymes isolated from rat liver and brain. No evidence was obtained to suggest the existence of a higher or lower molecular weight species having catechol-methyltransferase activity in any of these tissues at any stage of purification.

Chromatography of the solubilized microsomal enzymes from rat liver, heart and brain on Sephadex G-200 resulted in the enzyme activities eluting with the void volume indicating molecular weights in excess of 2×10^5 for these solubilized fragments.

Immunochemical studies

The interaction of the anti-rat liver catechol methyltransferase γ-globulin [40,41] with the catechol methyltransferases isolated from rat brain and heart was studied using both immunochemical and enzymatic methods. In Fig. 2 are shown the results of an immunodiffusion experiment where the antibody was placed in the center well and different amounts of the soluble catechol-methyltransferase isolated from rat liver, brain and heart were placed in the peripheral wells. A single continuous precipitin line of identity was observed. Immunoelectrophoretic examination of this cross reactivity between the soluble enzymes from heart and brain and the antibody also revealed only a single precipitin line.

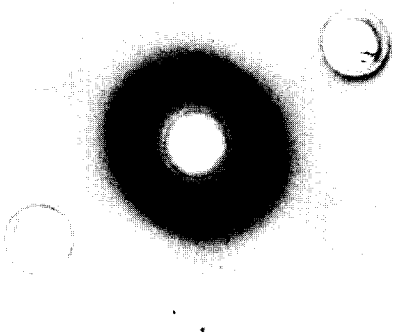


Fig. 2. Agar gel double-immunodiffusion reaction of anti-liver catechol-methyltransferase γ-globulin with purified soluble catechol-methyltransferase from rat liver, heart and brain. The center well contained the anti-liver catechol-methyltransferase γ-globulin and the peripheral wells going clockwise from 12 o'clock were filled with enzyme purified from the indicated sources: 9 μg liver enzyme, 24 μg heart enzyme, 28 μg brain enzyme, 18 μg liver enzyme, 48 μg heart enzyme and 14 μg brain enzyme. The volume per well was 10 μl and the plate was developed for 20 h at room temperature as described under Materials and Methods.

TABLE II

EFFECT OF ANTI-LIVER CATECHOL-METHYLTRANSFERASE γ-GLOBULIN ON CATECHOL-METHYLTRANSFERASE ACTIVITY ISOLATED FROM RAT HEART AND BRAIN

Experimental conditions consisted of preincubating (30 min, 25°C) a fixed amount of purified enzyme in phosphate buffer, pH 7.9 with varying amounts of the antibody. Residual enzyme activity was then determined using the assay conditions described in Table I. The anti-liver catechol-methyltransferase was obtained by immunizing New Zealand rabbits with purified rat liver enzyme as previously described [11,40,41].

Enzyme source *		% Inhibition of catechol methyltransferase activity with varying amounts of anti-liver catechol methyltransferase γ-globulin					
Tissue	Subcellular fraction	0 μl	10 μl	20 μl	30 μl	50 μl	100 μl
Brain	soluble	0 (0.164) **	44	70	74	85	92
	microsomal	0 (0.084) **	63	73	76	79	79
Heart	soluble	0 (0.350) **	38	71	76	81	89
	microsomal	0 (0.024) **	67	69	70	74	80

* Soluble enzymes were purified through the calcium phosphate step (See Table I). Microsomal enzyme refers to the resuspended pellet from the 100 000 × *G* centrifugation step.

** The specific activities of the control enzyme expressed as nmol of methylated product formed per mg of protein per min.

The cross reactivity of this anti-liver catechol-mehtyltransferase γ-globulin with the soluble and microsomal enzymes from rat brain and heart was also examined by testing the antibodies capability to inhibit the enzymatic reaction. In Table II is shown the effect of this antibody on the activity of soluble and microsomal catechol-methyltransferase isolated from rat heart and brain. When varying amounts of the antibody were added to constant amounts of the soluble heart or brain enzymes an increasing amount of inhibition of enzymatic activity was observed. The antibody also produced inhibition of the heart and brain microsomal enzymes. It is interesting to note that about 20% of the solubilized heart and brain microsomal activity appears to be resistent to inhibition by the antibody. Whether this reflects a catechol-methyltransferase-like species, which is unreactive with the antibody, or simply a residual activity of the antigen-antibody complex is unclear at this point.

Substrate specificity

In order to compare the specificity of the active sites of these enzymes, various methyl acceptors and methyl donors have been evaluated as substrates for the soluble enzymes from liver, heart and brain. The K_m and V values for some representative methyl acceptors are listed in Table III. The results suggest that no outstanding differences exist between these enzymes with respect to their specificity for the methyl acceptor. However, some minor differences do exist which might be physiologically important. For example, norepinephrine appears to be a better substrate for the liver and heart enzyme than for the

TABLE III

SUBSTRATE KINETIC PROPERTIES FOR SOLUBLE CATECHOL METHYLTRANSFERASE

The soluble enzymes were purified through the calcium phosphate gel step (Table I).

Substrate	Kinetic Constants					
	Liver		Heart		Brain	
	K_m ± S.E.M. (μm)	V ± S.E.M. ††	K_m ± S.E.M. (μm)	V ± S.E.M. ††	K_m ± S.E.M. (μm)	V ± S.E.M. ††
S-adenosyl-L-methionine *	9.66 ± 0.74	17.85 ± 0.46	28.1 ± 1.8	0.19 ± 0.01	53.7 ± 1.5	0.97 ± 0.01
S-3′-deoxyadenosyl-L-methionine *	337 ± 34	48.1 ± 3.1	70.3 ± 13.75	0.93 ± 0.15	69 ± 7	3.20 ± 0.21
S-tubercidinyl-L-methionine *	135 ± 15	23.8 ± 1.2	90 ± 12	0.19 ± 0.01	57.5 ± 11	0.24 ± 0.01
S-N^6-methyladenosyl-L-methionine *	348.5 ± 52	40 ± 4.0	56.3 ± 9	0.51 ± 0.03	39.5 ± 7	0.25 ± 0.01
S-3-deazaadenosyl-L-methionine *	635 ± 50	85 ± 5.1	73.5 ± 6.7	0.50 ± 0.02	124 ± 12	0.38 ± 0.02
S-8-azaadenosyl-L-methionine *	1170 ± 270	166 ± 31	623 ± 72	14.2 ± 4.1	268 ± 62	0.59 ± 0.08
3,4-dihydroxybenzoate **	320 ± 8.4	34.67 ± 4.51	159 ± 31	0.14 ± 0.01	103 ± 4.3	0.41 ± 0.01
3,4-dihydroxyacetophenone **	20.1 ± 5.2	1.30 ± 0.19	29.5 ± 1.5	0.06 ± 0.02	16.4 ± 2.0	0.26 ± 0.01
l-norepinephrine **	393 ± 43	13.67 ± 1.97	312 ± 81	0.19 ± 0.02	773 ± 116	0.20 ± 0.02
l-epinephrine **	553 ± 91	33.16 ± 2.89	346 ± 55	1.05 ± 0.07	225 ± 19	1.22 ± 0.04
Magnesium ion ***	— †	— †	266 ± 20	0.19 ± 0.01	212 ± 37	1.06 ± 0.07

* Assay conditions were similar to those outlined in Table I, except the methyl donor concentration was varied from 24 to 210 μM; dihydroxybenzoate concentration, 2.0 mM.

** Catechol substrate concentration variable; *S*-adenoxyl-L-methionine concentration, 1.0 mM.

*** *S*-adenosyl-L-methionine concentration, 1.0 mM; dihydroxybenzoate concentration, 2.0 mM; Mg^{2+} concentration variable.

† A K_m and V value for Mg^{2+} could not be obtained because of the nonlinear kinetics observed.

†† Expressed as nmol of methylated product formed per mg protein per min.

brain enzymes, whereas epinephrine has a lower K_m value with the brain enzyme than with the liver or heart enzymes. More dramatic differences were observed, however, in the methyl donor substrate specificity. The liver enzyme has the highest affinity (lowest K_m) for *S*-adenosyl-L-methionine whereas the brain and heart enzymes have much lower affinities (higher K_m values). Using various analogs of *S*-adenosyl-L-methionine synthesized earlier in our laboratory [34], we determined the structural features of this molecule which are needed to produce maximal methyl donor activity. The results listed in Table III would indicate that the liver enzyme has the highest specificity for the structural features of the methyl donor, since analogs such as *S*-3'-deoxyadenosyl-L-methionine, *S*,N^6-methyladenosyl-L-methionine and *S*-3-deazaadenosyl-L-methionine were all very poor substrates for the liver enzyme. In contrast, these analogs were all fairly good substrates for the heart and brain enzymes, suggesting these enzymes may have slightly different active sites than the liver enzyme.

The substrate specificities of the microsomal methyltransferases were also determined. Again very few differences in substrate specificity were observed between the microsomal enzymes isolated from rat heart, brain and liver. One possibly significant difference is the very high K_m values observed for norepinephrine as a substrate for liver microsomal enzyme (K_m = 1856 ± 734 μM) as compared to the heart (K_m = 169 ± 21 μM) and brain (K_m = 276 ± 59 μM) enzymes. It is also interesting to note that the liver microsomal enzyme (K_m = 68.7 ± 8.5 μM) has a substantially higher K_m value for *S*-adenosyl-L-methionine than the liver soluble enzyme (K_m = 9.66 ± 0.74 μM).

Inhibitor specificity

As another means of comparing the active sites of these enzymes, we have determined the inhibitory properties of various known inhibitors of liver soluble catechol-methyltransferase. The reversible inhibitors studied included tropolone, β-thujaplicin, 8-hydroxyquinoline, 3-hydroxy-4-pyridone, pyrogallol and 3,4-dimethoxy-5-hydroxybenzoic acid [2]. It was shown that all of these compounds effectively inhibited the soluble and microsomal enzymes isolated from rat liver, heart and brain. No significant differences were detected in the specificity of these enzymes for the inhibitors studied. In addition, each of these enzymes was inhibited equally well by *N*-ethylmaleimide and *p*-chloromercuribenzoate, indicating the existence of crucial sulfhydryl groups at the active sites of these enzymes [18].

In earlier studies from our laboratory [28—32], we had reported the inhibitory effects of various amino acid, base and sugar modified analogs of *S*-adenosyl-L-homocysteine on soluble rat liver catechol-methyltransferase. These analogs have proved to be useful probes to map the active site of this enzyme and to characterize the structural features of *S*-adenosyl-L-homocysteine which are needed to produce maximum enzymatic binding. Using these *S*-adenosylhomocysteine analogs we have examined here the binding requirements at the active sites of the heart and brain enzymes. Table IV shows the degree of inhibition of soluble heart and brain catechol-methyltransferase produced by these analogs of *S*-adenosyl-L-homocysteine. For comparison's sake, we have also listed the data for the soluble liver enzyme. It is apparent

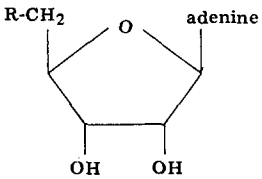
from the data listed in Table IV that the soluble heart and brain enzymes show specificities for the analogs of *S*-adenosyl-L-homocysteine similar to the soluble liver enzyme. These results would suggest that the soluble liver, heart and brain enzymes have similar binding sites for this natural inhibitor. We have also studied the kinetics of *S*-adenosyl-L-homocysteine inhibition of brain and heart catechol-methyltransferases. Consistent with data reported earlier for the soluble liver enzyme [28], we have observed that with the heart and brain enzymes, *S*-adenosyl-L-homocysteine exhibits competitive kinetics (liver, K_I = 36.3 ± 2.2 μM; heart, K_I = 39.7 ± 5.8 μM; brain, K_I = 21.9 ± 2.6 μM) when *S*-adenosyl-L-methionine was the variable substrate.

TABLE IV

INHIBITION OF RAT LIVER, HEART AND BRAIN CATECHOL-METHYLTRANSFERASE BY ANALOGS OF *S*-ADENOSYL-L-HOMOCYSTEINE

The soluble enzymes were purified through the calcium phosphate step. Assay conditions were the same as those described in Table I except that inhibitor concentration was 0.2 or 2.0 mM.

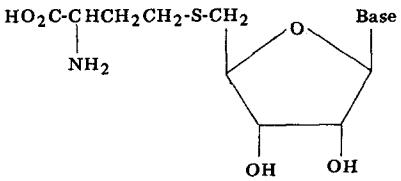
Amino acid modifications:



Compound	R=	Inhibitor concn. (mM)	% inhibition		
			Liver	Heart	Brain
L-SAH	-S-$CH_2CH_2CH(NH_2)CO_2H$(L)	0.2	39	19	26
		2.0	87	93	90
D-SAH	-S-$CH_2CH_2CH(NH_2)CO_2H$(D)	0.2	5	5	7
		2.0	22	24	20
L-SAHO	-S-$CH_2CH_2CH(NH_2)CO_2H$	0.2	14	23	3
	↓ O	2.0	42	43	43
L-$SAHO_2$	-S-$CH_2CH_2CH(NH_2)CO_2H$	0.2	8	23	0
	↓↓ O O	2.0	45	79	25
L-SAC	-S-$CH_2CH(NH_2)CO_2H$	0.2	0	1	2
		2.0	5	12	10
Decarboxylated SAH	-S-$CH_2CH_2CH_2NH_2$	0.2	1	0	0
		2.0	6	7	0
Deaminated SAH	-S-$CH_2CH_2CH_2CO_2H$	0.2	4	0	0
		2.0	8	6	10
N^{α}-acetyl-L-SAH	-S-$CH_2CH_2CH(NAc)CO_2H$	0.2	1	0	0
		2.0	10	7	0

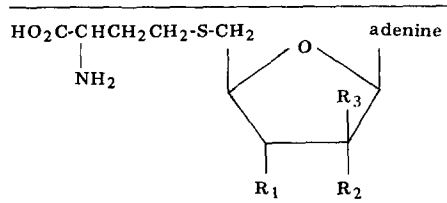
Table IV (continued)

Base modifications:



Compound	Base	Inhibitor concn. (mM)	% inhibition		
			Liver	Heart	Brain
SGH	Guanine	0.2	0	0	0
		2.0	0	7	9
SIH	Hypoxanthine	0.2	2	2	0
		2.0	16	8	15
SUH	Uracil	0.2	0	0	0
		2.0	10	7	7
SCH	Cytosine	0.2	0	0	0
		2.0	5	11	10
N^6-methyl-SAH	N^6-methyladenine	0.2	15	31	5
		2.0	55	58	46
N^6-dimethyl-SAH	N^6-dimethyl-adenine	0.2	6	0	0
		2.0	18	27	30
3-deaza-SAH	3-deazaadenine	0.2	25	28	12
		2.0	68	46	43
N^6-methyl-3-deaza-SAH	N^6-methyl-3-deazaadenine	0.2	9	8	0
		2.0	18	17	6
N^6-dimethyl-3-deaza-SAH	N^6-dimethyl-3-deazaadenine	0.2	1	19	10
		2.0	5	47	53
8-aza-SAH	8-azaadenine	0.2	5	12	0
		2.0	28	52	47
STH	7-deazaadenine	0.2	56	59	58
		2.0	94	100	100

Sugar modifications:



Compound	R_1	R_2	R_3	Inhibitor concn. (mM)	% inhibition		
					Liver	Heart	Brain
2'-deoxy-SAH	OH	H	H	0.2	0	0	0
				2.0	2	19	15
3'-deoxy-SAH	H	OH	H	0.2	22	0	0
				2.0	59	31	48
SAraAH	OH	H	OH	0.2	0	3	6
				2.0	0	14	13

* Expressed as percent inhibition of the O-methylation of 3,4-dihydroxybenzoate.

Discussion

Soluble catechol-methyltransferase from rat liver has been purified and its properties characterized by various laboratories [1—20]. Less extensive investigations have been carried out on the catechol-methyltransferase activities in rat brain [12,42] and heart. In this paper we have reported the purification of both the soluble and microsomal forms of catechol-methyltransferase present in these tissues. We have also carried out an extensive investigation of the properties of these enzymes (molecular weights, substrate specificities, inhibitor specificities, immunochemical properties, etc.) in an effort to determine whether the proteins are identical or whether they may be isozymes having slightly different physical and chemical properties. The results of this study suggest that only a single form of soluble catechol-methyltransferase exists in these tissues. These results contradict those of Marzullo and Friedhoff [13], who have reported two different forms of catechol-methyltransferase in rat liver. In this study we have carefully searched for higher molecular weight forms of this enzyme without success. Each of the tissues examined have an enzyme with a molecular weight of 23 000 and all possess similar chemical, physical and immunochemical properties. The soluble enzymes in each of these tissues require a catechol substrate, *S*-adenosylmethionine and magnesium for activity. Each of the enzymes are stabilized and the catalytic activity stimulated by sulfhydryl compounds (e.g. dithiothreitol). The enzymes also show immunological identity, since the soluble catechol-methyltransferases from heart and brain each react with an antibody generated against the liver soluble enzyme. In immunodiffusion and immunoelectrophoretic experiments only a single precipitin line was observed for the reaction of the antibody with the enzyme purified from these tissues.

In this study we have attempted to map the substrate binding sites on these enzymes by carefully examining their substrate and inhibitor specificities. This was accomplished by evaluating various catechols as methyl acceptor substrates and various analogs of *S*-adenosylmethionine as methyl donors. We have also evaluated known reversible and irreversible inhibitors of the rat liver enzyme for their abilities to inhibit the soluble heart and brain enzymes. It can be generally concluded that the catechol binding sites on these enzymes are quite similar, since ligands which have the potential to bind at this site (e.g. catechol substrates or dead-end inhibitors) exhibit similar binding characteristics with all three enzymes. Some minor differences do exist, but none sufficiently significant to suggest substantial differences in the catechol binding sites on these enzymes. We have also used analogs of *S*-adenosylhomocysteine to map the methyl donor binding sites. The results of the studies would suggest that no significant differences exist in the *S*-adenosylhomocysteine binding sites. However, the results obtained using analogs of *S*-adenosyl-L-methionine as methyl donors would suggest slight differences in the catalytic transition states for methyl transfer process. The soluble liver enzyme exhibited stricter methyl donor specificity, suggesting perhaps a 'tighter' transition state for the methyl transfer catalyzed by this enzyme. In contrast the heart and brain enzymes showed less stringent specificity for the methyl donor suggesting a more 'flexible' transition state. This proposed difference in the transition state

is the most reasonable explanation for the differences observed between these enzymes in their methyl donor specificity. Since similar differences were not observed in the inhibitory effects of the analogs of *S*-adenosylhomocysteine, differences in binding requirements alone could not explain the effects observed with the analogs of *S*-adenosylmethionine.

From the studies using the solubilized preparations of liver, brain and heart microsomal catechol-methyltransferase, it can be generally concluded they exhibit similar properties to the soluble enzymes, including substrate specificities, inhibitor specificities, pH optima and partial immunological identities. The observation that a portion of the solubilized microsomal enzyme from brain and heart is resistant to the inhibition by the antibody to soluble liver enzyme may result from the fact that the enzyme is still attached to membrane fragments of varying size with perhaps various means of attachment to the membrane fragments. Depending on the nature of the attachment, some of the antibody-antigen specificity may be lost, since attachment to the membrane may be through or near the antigenic site.

Therefore, in conclusion we feel that the properties of both the soluble and microsomal forms of catechol-methyltransferase present in rat brain, liver and heart are similar, and if differences do exist they are minimal, perhaps resulting from slight differences in the secondary or tertiary structures rather than the primary structures of these proteins.

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