SOME PROPERTIES OF THE ORGANOMERCURY-DEGRADING SYSTEM IN MAMMALIAN LIVER

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1. Introduction

The presence of a system capable of catalysing the liberation of inorganic mercury from a variety of organomercury compounds has recently been detected in the soluble fraction of rat liver [1] and in cell-free extracts of a mercury-resistant pseudomonad [2]. The latter complex, which has been shown to consist of a 'mercury-degrading enzyme' together with cytochrome c_1, has an absolute requirement for NAD(P)H and the product of the reaction has been identified as elemental mercury (Hg^0). The mammalian system does not catalyse the formation of Hg^0 and has been represented as an hydrolysis. We now wish to present preliminary data concerning some of the properties of the system present in rat and guinea-pig liver.

2. Methods

Tissues were homogenised at 4°C in 3 vol 0.1 M Na_2HPO_4–KH_2PO_4 buffer (pH 7.4) using 5 passes of a teflon-in-glass homogeniser. After centrifugation at 200 000 g_{av} for 40 min at 4°C, the supernatant was collected and either used immediately or stored at −20°C until required. Supernatant, equivalent to 20 mg protein, was incubated for 30 min at 37°C in a final volume of 5 ml with approx. 10^{-3}–10^{-4} M (0.2 mg) of the appropriate organomercurial. The flask was sealed with a rubber-diaphragm stopper. Inorganic mercury was determined after reduction [3] to Hg^0 by flameless atomic absorption. Protein was estimated by the biuret reaction [4]. Benzene and ethylene were identified as products of the reaction of phenylmercury acetate (PMA) and methoxyethylmercury chloride (MEMC) by GLC-analysis of portions (1 ml) of the vapour phase [1, 5]. Benzoic acid was identified by GLC-analysis of the methyl-ester on a column (7 ft × $\frac{1}{4}$ in) of Silicone E301 (5%) on Chromasorb W. The retention time of methyl benzoate was 230 sec under the operating conditions used (column temperature 98°C; N_2 flow-rate 75 ml min^{-1}).

3. Results and discussion

The activity of the system in the soluble fraction of liver (L), brain (B) and kidneys (K) of a number of species is shown in table 1. PCMB was rapidly degraded when incubated with rat, mouse and guinea-pig liver and with rat brain. Little activity was detected in the livers of either the ferret or chicken. There was little species variation in activity when either PMA or MEMC were used as substrates. The variation in the activity in the liver preparations toward all three substrates suggests the presence of at least two systems capable of effecting cleavage of the C–Hg bond. The values of K_m for PMA (0.07 mM) and PCMB (0.04 mM) (fig. 1a) as well as MEMC (0.03 mM) were obtained by the method of Dixon [6]. Ethylene, benzene and benzoic acid were identified as products of the reaction with MEMC, PMA and PCMB, respectively. No evidence was obtained for the formation of Hg^0 in any of the experiments and no inorganic mercury was formed when either methylmercury, ethylmercury or mersalyl was incubated with preparations from rat or guinea-pig liver. This specificity is in marked contrast with that observed in the microbial system [2].

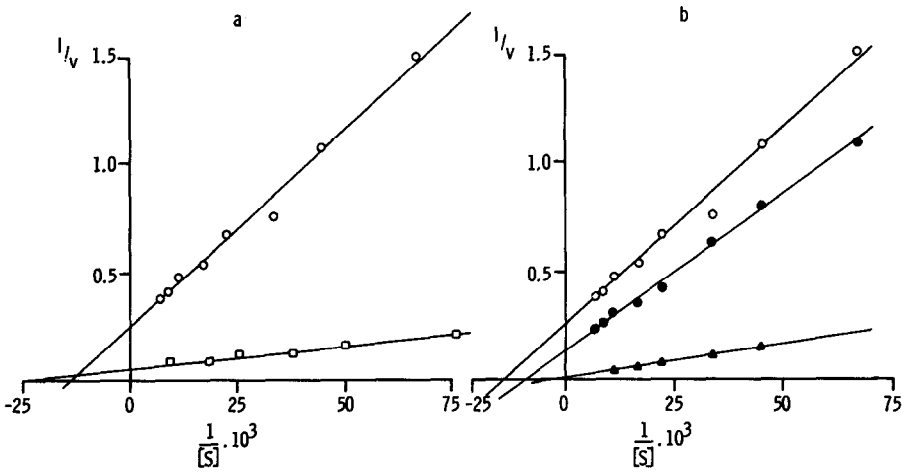

Fig. 1. Lineweaver–Burk plots for the action of guinea-pig liver soluble fraction a) with either PMA (o—o—o) or PCMB (□—□—□) as substrate; b) the effect of DTT at 10^{-4} M (▲—▲—▲); 10^{-5} M (●—●—●) or zero concentration (o—o—o) on the degradation of PMA.

Approx. 25–35% of the PMA-degrading activity of rat and guinea-pig liver supernatants is lost during storage for 18 hr at 4°C. No loss occurred if the fractions were stored at −20°C. In an attempt to protect against the loss of activity observed at 4°C the effects of adding several thiol-compounds were examined. The addition of dithiothreitol (DTT; 10^{-3} M) to rat liver supernatant stimulated PMA-degrading activity 10-fold, while the effect on the degradation of MEMC was less pronounced (3-fold stimulation). Stimulation was also observed at concentrations of DTT of 10^{-6} M. This effect of DTT on the rate of degradation of PMA was associated with a change in the value of both V_{max} (from 0.38 to 16.2 μg Hg^{2+} liberated/mg protein in the presence of 10^{-4} M DTT) and K_m (fig. 1b). No explanation can be offered which could account for this effect, nor for the apparent dependence of K_m on the DTT concentration. The addition of GSH, L-cysteine and thioglycollate, each at 10^{-3} M, produced variable inhibition (30–50%) of activity; 2-mercaptoethanol (10^{-3} M) produced a 2-fold stimulation in the PMA-degrading activity. The addi-

Table 1
The activity of the organomerury degrading system in the tissues of various species.

Species	PMA			MEMC			PCMB		
	L	B	K	L	B	K	L	B	K
Rat	83	84	51	36	42	52	1795	2663	19
Mouse	74	–	–	35	–	–	581	–	–
Guinea-pig	115	37	37	101	–	26	1760	–	53
Ferret[1]	133	58	29	34	–	25	18	–	–
Chicken[1]	34	–	21	12	–	10	9	–	3

Results are the average of 5 or more experiments and are expressed as ng Hg^{2+} liberated/mg protein/30 min.
⁻: Not determined.
* Single animals only.

Table 2
$(NH_4)_2SO_4$ fractionation of rat and guinea-pig post-microsomal supernatant.

%$(NH_4)_2SO_4$ saturation	Rat		Guinea-pig	
	Activity recovered (%)	Specific activity*	Activity recovered (%)	Specific activity*
20–40	5.9	57	8.8	42
40–60	16.9	56	40.8	139
60–80	8.3	80	6.8	64
Total	31.1		56.4	

* Results expressed as ng Hg^{2+} liberated/mg protein/30 min, using PMA as substrate.

Table 3
Ultrafiltration of the soluble fraction of rat and guinea-pig liver.

Fraction	Activity*			
	Rat		Guinea-pig	
Original supernatant	100	100	100	100
Retentate (R)	55	78	93	107
Filtrate (F)	80	93	0	0
R + F	53	81	89	97

* Activity expressed as a percentage of that found in the original supernatant.

tion of *N*-ethylmaleimide (10^{-3} M) to the incubation medium resulted in a 40–50% loss of activity while iodoacetate (10^{-2} M) abolished all activity.

Two procedures were used in an attempt to fractionate the components of rat and guinea-pig liver. The soluble fraction was treated with ammonium sulphate, the precipitates redissolved in 0.1 M phosphate buffer (pH 7.4) and dialysed against buffer for 18 hr at 4°C. As shown in table 2, when PMA was used as the substrate, activity was found in all three fractions, although most was present in F2. There was some evidence for a slight increase in the specific activity in F2 from guinea-pig liver but not from rat liver. Substantial amounts of activity were lost using this procedure. The activity of all three fractions was stimulated equally in the presence of DTT (10^{-3} M).

The soluble fractions were therefore subjected to ultrafiltration using an Amicon micro-ultrafiltration system, Model 8MC, incorporating a membrane which retained all material with a molecular weight greater than 10^4. With rat liver, PMA degrading activity was present both in the filtrate and in the retained material. When the filtrate and retentate were combined, however, the activity obtained approximated that of the retentate alone (table 3). In contrast, no activity was detected in the filtrate of guinea-pig liver supernatant. The data suggest the presence, at least in rat liver, of both a high and a low molecular weight component which can both catalyse the liberation of inorganic mercury from PMA.

4. Summary

The liver, brain and kidneys of rats and guinea pigs possess the ability to degrade a variety of organomercury compounds to inorganic mercury. The PMA-degrading activity of the hepatic system is markedly stimulated by the addition of dithiothreitol or 2-mercaptoethanol but not of other sulphydryl compounds. Fractionation of the components in the soluble fraction of rat liver indicates the presence of a high and a low molecular weight component, the effects of which are non-additive. Only a high molecular weight component was detected in guinea-pig liver.

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