OCCURRENCE AND SOME PROPERTIES OF A MERCURI-ACTIVATABLE AMINO ACID ESTERASE FROM RAT KIDNEY MICROSOMES

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1. Introduction

Our previous studies on the properties of the mammalian kidney esterases have shown the presence of an amino acid esterase (TEE-ase) in rat kidneys which was stimulated by the sulfhydryl reagent *p*-chloromercuribenzoate (*p*-CMB) [1, 2]. Further experiments revealed that this *p*-CMB activatable TEE-ase belongs to the B-type esterase group with microsomal origin and was restricted to rat kidney because the kidney TEE-ase of other species (e.g. rabbit, guinea pig, mouse, bovine, porcine, human) proved to be unaffected by the same *p*-CMB concentrations (0.05–1 mM) which caused a stimulation of the rat enzyme [1, 3]. Continuing our studies on the possible physiological function of this unusual rat kidney enzyme it was desirable to investigate its properties in more detail.

In this paper we show that the activation effect given by various SH-reagents was organ- and substrate-specific, time dependent, noncompetitive in nature, and consisted in the decrease of activation energy of the esterolytic action on amino acid esters.

Our results on the in vivo-effect of *p*-CMB on the TEE-ase and on comparative studies of the untreated and *p*-CMB-treated enzyme are to be published elsewhere [4, 5].

2. Materials and methods

Etherized male adult Wistar rats (250–300 g) were sacrificed by cardiac puncture. The blood free saline- and sucrose-perfused kidneys were minced in ice cold 0.25 M sucrose (pH 7.0) in a ground glass Potter–Elvehjem homogenizer. 10% (w/v) Homogenates were used routinely. After removing nuclei, cell debris and mitochondria, microsomes were isolated by centrifuging the resulting supernatant for 90 min at 40 000 *g* in a MSE refrigerator centrifuge. To solubilize the particle-bound TEE-ase in high yield the microsomes were lysed with 0.3% Triton X-100 in M/15 phosphate buffer, pH 7.0 and subsequently free from the inactive pellets by centrifugation at 30 000 *g* for 30 min. The kidney and liver homogenates of the other species tested (guinea pig, mouse, rabbit) were prepared in an analogous manner.

The amino acid esterase was determined either by quantitative paper chromatography at pH 8.4 [6] or by means of the Ph-stat technique at pH 9.1–9.2 and 37.5°C using L-tyrosine (TEE)-, L-phenylalanine(PEE)- or L-leucine(LEE)-ethyl ester, L-tyrosine propyl ester (TPE), and *p*-hydroxyphenyl propionic acid ethyl (HPPEE) as substrate [7]. The non-specific esterase activity was determined with phenyl-acetate (PA), -propionate (PP), or -butyrate (PB) as substrate at pH 8.0 in the pH-stat equipment. For further details see [1] and [7].

Protein was estimate by the Lowry method [8].

The following sulfhydryl reagents were used: *p*-chloromercurybenzoate (*p*-CMB) (Koch and Light, England), $HgCl_2$ and iodoacetamide (IAA) (VEB Laborchemie, Apolda), and *N*-ethylmaleimide (NEM) (ARCO Chemie, Berlin). 2-Mercaptoethanol (ME) was purchased from Fluka AG, Basel.

If not otherwise stated the enzyme preparation was pretreated with the corresponding effector for 10 min at room temperature and pH 8.0–8.5. The esterolytic

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reaction was initiated by addition of the appropriated substrate solution.

3. Results

In addition to the reported species-specific stimulation effect of *p*-CMB, $HgCl_2$, and other SH-reagents on the rat kidney TEE-ase [3] this enzyme was organ-specific in nature because the TEE-ase of other organs of the rat e.g. liver and serum proved to be uneffected by *p*-CMB (fig. 1). Compared with crude homogenate the activation effect of *p*-CMB observed with the microsomal extract is higher and more circumscribed (fig. 1). The reason for the lack of activation by $HgCl_2$ in the 10^{-3} M range may lie in the pronounced protein denaturation effect of Hg^{2+}.

The stimulation effect was detectable with all amino acid esters tested, e.g. TEE, TPE, PEE, LEE, tryptophaneEE, and with the amino group free TEE analogue HPPEE. On the other hand the hydrolysis of

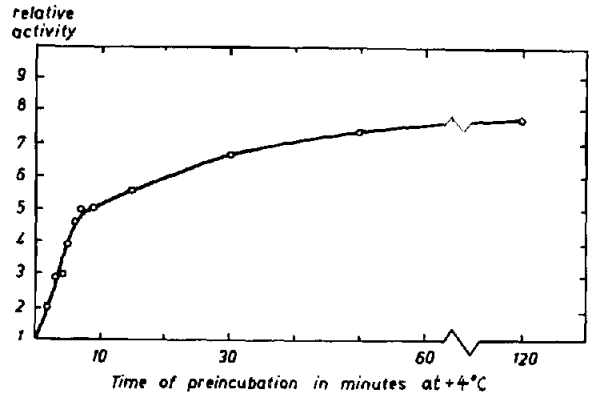

Fig. 2. Activation of the microsomal amino acid esterase by 0.125 mM *p*-CMB at +4° C in dependence of time. For further details see fig. 1.

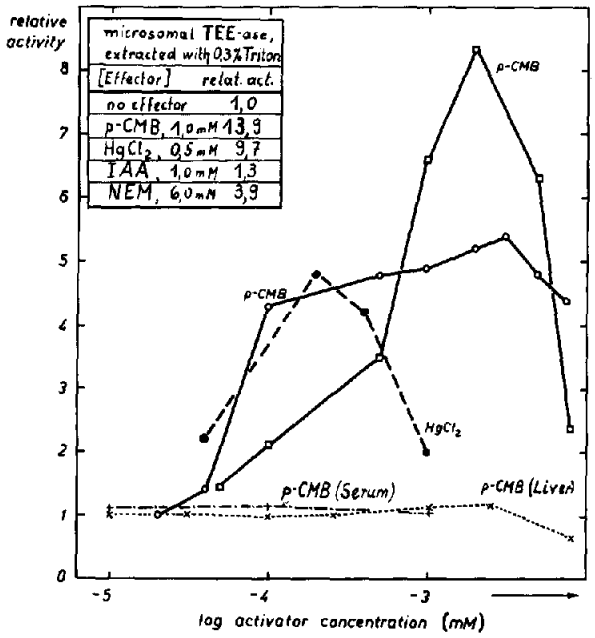

Fig. 1. Activatibility of amino acid esterase from the kidney, liver and serum of the rat by different concentrations of *p*-CMB and $HgCl_2$ (the latter for kidney only). (○—○—○) Crude homogenate of kidney; (□—□—□) microsomal enzyme. In the inset the relative maximal activation effects of 4-SH-reagents on the Triton X-100 extracted microsomal amino acid esterase are summarized. Substrate, 0.02 M L-tyrosine ethyl ester (TEE), pH 9.15, pH-stat technique.

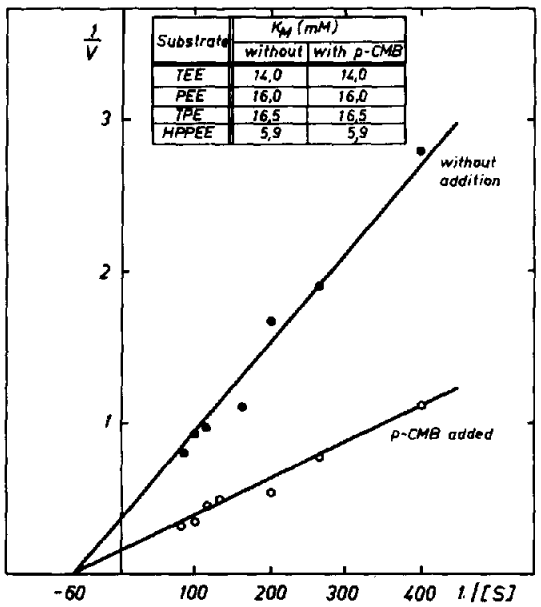

Fig. 3. Lineweaver–Burk plot of the *p*-CMB-activation effect on microsomal amino acid esterase. (Substrate: TEE). The inset comprises the K_M values for 4 different substrates in absence and presence (0.125 mM) of *p*-CMB.

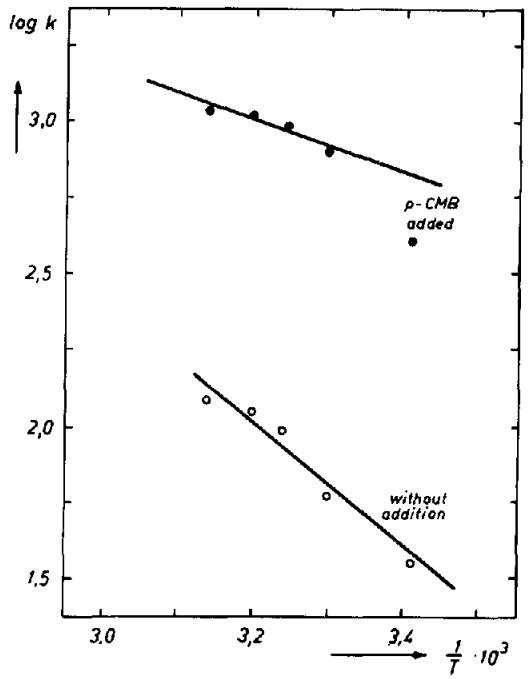

Fig. 4. Arrhenius plot of the untreated and *p*-CMB-treated enzyme. Substrate, 0.02 M TEE, pH 9.15, pH-stat technique.

typical carboxylic esters such as PA, PP, PB, ethyl butyrate of triacetin by kidney microsomes was reduced in presence of *p*-CMB. The increasing effect on TEE-ase is not only given by *p*-CMB and $HgCl_2$ but also by other SH-blocking agents such as NEM and IAA (see inset of fig. 1).

The optimal effector concentration for the activation of microsomal TEE-ase was found to be 2–3 mM for *p*-CMB and 0.2 mM for $HgCl_2$ (fig. 1).

The half maximal efefct of activation was estimated to be 0.3 mM and 0.08 mM for *p*-CMB and $HgCl_2$ respectively. The Hill-coefficient is $n = 1.06$ in respect to *p*-CMB.

The time dependence of the activation at 4°C by *p*-CMB is shown in fig. 2. Whereas half maximum activity was reached after seven minutes already, full activation needs several hours of incubation with *p*-CMB. At room temperature the same effect was attained after 3–4 min and 2 hr respectively.

The activation is reversible because addition of ME in amounts equal to *p*-CMB after reaching maximal stimulation abolish this effect. At higher concentrations ME inhibits TEE-ase.

As can be seen in fig. 3 the activating effect of *p*-CMB on the TEE-ase is of a noncompetitive type since the K_M value remains unchanged. The same is true for the hydrolysis of the other amino acid esters whose K_M values are listed in the inset of fig. 3.

The effect of temperature on the TEE-ase was determined by assaying the enzyme activity at various temperatures in absence and presence of *p*-CMB. In both cases maximum of activity was found to be near by 45°C. Fig. 4 shows an Arrhenius plot of the data measured at various temperatures. The most striking result besides the above mentioned enhancing effect is that *p*-CMB causes a significant decrease in activation energy of the TEE-ase from 9.2 kcal/mole to 3.8 kcal/mole in the temperature range 30 to 40°C. At lower temperatures however energy of activation of the *p*-CMB treated enzyme was close by that of the untreated enzyme.

4. Discussion

In 1958 Hess and Pearse [9] first provided evidence for the occurrence of a phenylmercurichloride activatable indoxylacetate esterase in the tubuli of rat kidney by histochemical techniques. Our results indicate that rat kidney microsomes contain an amino acid esterase activatable by mercurials and other sulfhydryl reagents. This result implies that sulfhydryl groups may be involved which are accessible by reagents reacting either by mercaptide formation (*p*-CMB, $HgCl_2$), alkylation (IAA) or by addition (NEM). The reason for this unexpected stimulation effect of the SH-reagents so far known as enzyme inhibitors can be tentatively attributed to changes in conformation of the modified enzyme. The unaltered K_M value and the lowered energy of activation of the mercuri-enzyme may support this assumption. Since the TEE-ase consists of three subunits [5] alterations in subunit interaction could be involved too. Probably the detected isoenzyme nature of purified TEE-ase [5] may also play some as yet unknown role during this activation process.

Similar effects of *p*-CMB on enzyme accompanied by a non-competitive activation was shown for adenosine deaminase [10] and malate dehydrogenase [11], including a lowered activation energy for the former *p*-CMB-treated enzyme. Beyond this there have been shown other enzymes also known to be activated by SH-reagents but only to a smaller extent [12–20].

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