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SHORT COMMUNICATIONS

Enzymatic defluorination of methoxyflurane

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Methyoxyflurane (2,2-dichloro-1,1-difluoroethyl methyl ether [MOF]) is an anesthetic which produces biochemical and histological changes in man and experimental animals [1–14]. Its metabolic products include inorganic fluoride and chloride, carbon dioxide, oxalic acid and acetic acid derivatives [15–22]. The available data indicate that MOF is defluorinated and dechlorinated by a microsomal-bound mixed function oxidase system [17, 23, 24]. Contrary to a report by Van Dyke and Wood [24], we have found that the soluble fraction of rat liver extracts contains enzymatic material which produces defluorination of MOF.

Livers from male Wistar rats were homogenized at 4° in 3 vol (ml/g) of 0.15 M Tris–acetate buffer (pH 8.5). The suspension was centrifuged for 20 min at 9000 *g*, and the supernatant was centrifuged at 105,000 *g* for 60 min. The supernatant from the final centrifugation provided the 'soluble fraction'. Microsomes were obtained by homogenization in 1.15% KCl and centrifugation as above [24]. Part of the microsomal pellet was resuspended in 0.05 M Tris–chloride buffer (pH 7.4) to test its aniline hydroxylase [25] and *O*-demethylase [26] activities, and the remainder was added to the soluble fraction in 0.15 M Tris–acetate (pH 8.5) to test for alteration of MOF defluorination activity under conditions in which the soluble fraction produced maximum activity. Protein was measured by the method of Lowry *et al.* [27].

All components of reaction mixtures, with the exception of MOF, were added to glass serum bottles (dia 5 cm, height 7.5 cm, vol 120 ml). Each bottle was sealed with a rubber stopper and flushed with a gas transmitted by needles through the stopper. MOF was introduced by syringe and needle to start the reaction (final vol 10 ml, 37°), and 0.5-ml aliquots were removed at intervals and diluted in 0.5 ml of TISAB.* Fluoride was measured at pH 5.4 with an Orion model 407 ion-specific meter utilizing a model 96-09 fluoride-specific electrode standardized against known sodium fluoride concentrations prepared in 0.15 M Tris–acetate buffer and measured in TISAB at pH 5.4.

The soluble fraction of rat liver extracts contained material which produced defluorination of MOF at a constant rate for at least 21 hr in a relatively anaerobic atmosphere (Fig. 1). Preheating of the soluble fraction or preincubation with trypsin destroyed the enzymatic activity. Maximum activity was obtained at pH 8.5 (Fig. 2) and at addition of 1.3 m-moles of MOF per 10 ml of reaction mixture. The enzyme activity was not altered when the soluble fraction was preincubated at temperatures of 50° or less, but activity was partially destroyed by preincubation at 55° for 10 min and was completely destroyed by preincubation at 60° for 10 min (Fig. 3). Addition of microsomes (prepared at pH 7.4 in 1.15% KCl and shown to

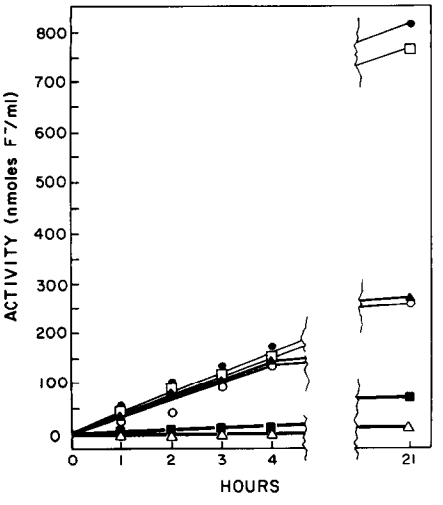

Fig. 1. Catalysis of MOF defluorination by the soluble fraction of rat liver homogenates. Gas phases used: (●) argon; (□) nitrogen; (▲) oxygen; (○) air; (■) argon used with soluble fraction which had been heated at 60° for 10 min; (△) argon used with soluble fraction which had been preincubated with trypsin (0.8 mg/ml) at 25° for 16 hr. Controls for the trypsin digestion, including addition of trypsin inhibitor prior to trypsin, were fully active. Protein concentrations were 17 mg/ml.

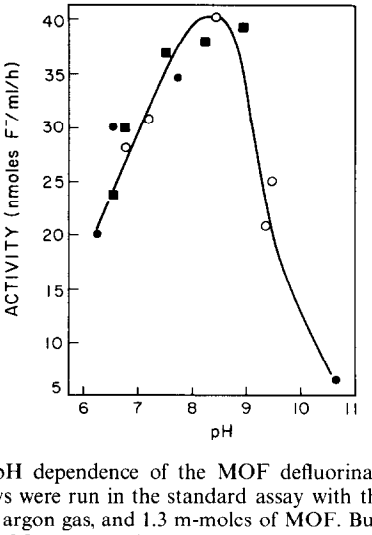

Fig. 2. pH dependence of the MOF defluorination rate. All assays were run in the standard assay with the soluble fraction, argon gas, and 1.3 m-moles of MOF. Buffers were all at 0.1 M concentrations; (●) potassium phosphate; (○) sodium glycinate; (■) sodium glycylglycinate. Protein concentrations were 21 mg/ml.

* TISAB is a solution of sodium chloride (58 g), cyclohexylene dinitrilotetraacetic acid (4 g) and glacial acetic acid (57 ml) dissolved in water and sodium hydroxide to a volume of 1 liter, with a pH between 5.0 and 5.5 (Orion Research Inc., Form 1 M 96, 94-09/375, p. 9, 1973).

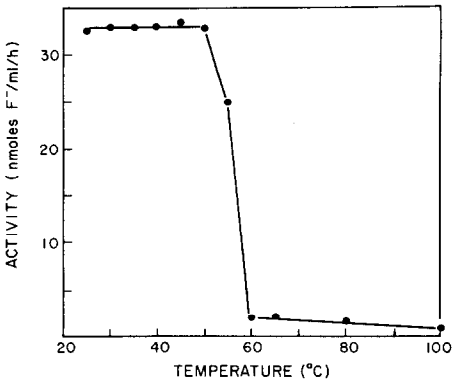

Fig. 3. Temperature stability of MOF defluorinating enzyme. The soluble fraction was heated at the specified temperature for 10 min; MOF (1.3 m-moles) was added; and defluorination under argon was followed at 37° for 4 hr. The protein concentrations were 23 mg/ml.

be active with aniline and *p*-nitroanisole) did not alter the activity when added to the soluble fraction. Microbial contamination was excluded as a possible source of enzymatic activity, since reactions carried out in test solutions which contained penicillin G (20 units/ml) and streptomycin sulfate (20 μg/ml) were fully active and were sterile for at least 24 hr.

A preparation of the soluble fraction was divided into two portions, one of which was dialyzed against Tris–acetate buffer to provide a high-molecular-weight (high-mol-wt) fraction in the retentate. The second portion was filtered through an Amicon PM-30 membrane to provide a low-molecular-weight (low-mol-wt) fraction in the ultrafiltrate. The high-mol-wt fraction contained only 30–45 per cent of the original activity, and the low-mol-wt fraction was completely inactive (Table 1). Complete recovery of MOF defluorinating activity was obtained by recombination of the two fractions. The activity of the recombined system was lost when the high-mol-wt fraction was heated at 60° for 10 min.

Enfurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether), a structural analog of MOF, was not defluorinated in this soluble system. It was also ineffective in inhibiting MOF defluorination when tested against rate-limiting amounts of MOF.

Whether fluoride is the primary product of the enzymatic attack upon MOF is not known. It is equally possible that the enzymatic system produces a dechlorinated intermediate, which subsequently loses fluorine by hydrolysis.

Table 1. Defluorination of MOF by the high- and low-molecular-weight fractions obtained from the soluble fraction of rat liver extracts

Fraction	Activity*	Per cent
Complete soluble	1.71 ± 0.10	100
High-mol-wt	0.64 ± 0.12	37
Low-mol-wt	0	0
High- and low-mol-wt recombined	1.67 ± 0.15	98

* Activity of the soluble fraction is nmoles F^- released/mg protein/ml/hr. Activities of the other fractions are computed relative to the amount and activity of the soluble fraction from which the high- and low-mol-wt fractions were derived. The results are tabulated from three separate experiments.

The high endogenous levels of chloride in rat liver extracts has not permitted us to study dechlorination, and the answer to this question, as well as the identification of other reaction products, must await further resolution of the enzyme system.

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Division of Laboratories and Research, WILLIAM A. WARREN
New York State Department of Health, FREDERICK D. BAKER
Albany, New York 12201 *U.S.A.* JANIS BELLANTONI

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