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Original Scientific Paper

Hydrolysis of Some Monomethylcarbamates in Human Sera*

E. Reiner and M. Škrinjarić-Špoljar

Institute for Medical Research, Yugoslav Academy of Sciences and Arts, Zagreb, Croatia, Yugoslavia

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The rate of hydrolysis of 1-naphthyl-*N*-methylcarbamate (sevin), 3-isopropylphenyl-*N*-methylcarbamate (IPFC) and 2-isopropoxyphenyl-*N*-methylcarbamate was measured in sera of 12 individuals. Sevin was hydrolysed 3.1 times faster than IPFC, while 2-isopropoxyphenyl-*N*-methylcarbamate did not hydrolyse under given experimental conditions. The Michaelis constants (K_m) and the maximal rates of hydrolysis (*V*) of sevin and IPFC were determined. Both, K_m and *V* varied from one serum to the other. For each serum V_{sevin} was 2.9 times higher than V_{IPFC}. The Michaelis constants for both compounds varied within the same range.

Sevin and IPFC were not hydrolysed by the serum fraction IV—1. Serum fraction V hydrolysed sevin approximately 4 times faster than IPFC. Native serum and serum fraction V did not alter their activity upon incubation at 53° C up to 3 and 6 hr., respectively. It is suggested that the same enzyme(s) hydrolyse sevin and IPFC in human sera.

Plasmata of various species are known to hydrolyse esters of carbamic acids[1], but little is known about the enzymes participating in these reactions. The hydrolysis of 1-naphthyl-*N*-methylcarbamate in human serum was shown to be associated with the albumin fraction[2] while the hydrolysis of *N,N*-dimethylcarbamoyl fluoride occurred primarily in the serum fraction IV-1[3].

In the present paper are given the rates of hydrolysis of three monomethylcarbamates in human sera and in two serum fractions — fraction IV-1 and fraction V. Heat inactivation studies are included in the paper. The Michaelis constants and the maximal rates of hydrolysis were determined for native human sera and two of the compounds studied.

MATERIALS AND METHODS

The enzyme source was human serum obtained from defibrinated blood of 12 individuals (3 male an 9 female); the serum fractions were obtained from the Institute of Immunology, Zagreb, Yugoslavia (fraction V) and from AB Kabi, Stockholm, Sweden (fraction IV-1). Stock solutions of the fractions were prepared in buffer (75 mg./ml. for fraction V; 25 mg./ml. for fraction IV—1). 1-Naphthyl-*N*-methylcarbamate (sevin), 3-isopropylphenyl-*N*-methylcarbamate (IPFC) and 2-isopropoxyphenyl-*N*-methylcarbamate were used as substrates; stock solutions (0.1 *M*) were prepared in ethanol with 10% Tween-80, and suitably diluted with buffer immediately before use. All experiments were done in phosphate buffer 0.05 *M*, *p*H 8.0, at 37° C; the buffer contained 0.1% Tween-80. The rate of substrate hydrolysis was measured spectrophotometrically with the 4-aminoantipyrine reagent for phenols[2]. During substrate hydrolysis the final serum concentration was kept at 0.05% (v/v), while the

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substrate concentration varied from 0.1 to 0.8 m*M*; the time of hydrolysis was 20 min. The concentration of fraction IV—1 was 1.0 mg./ml. and that of fraction V varied from 1.0 to 5.0 mg./ml.; the time of hydrolysis varied from 5 to 60 min. The final ethanol concentration during substrate hydrolysis was 1.0%, and that of Tween-80, 0.1%. The extinction was read in 1.0 cm. cells, at 510 mμ, on a Unicam spectrophotometer model SP 600. The activities were expressed in μmoles hydrolyzed substrate per min. per ml. serum or mg. of serum fraction. All activities were corrected for spontaneous hydrolysis of substrates.

Heat inactivation studies were done at 53° C. Undiluted serum or stock solutions of fraction V were kept at 53° C; at suitable time intervals samples were withdrawn and the activities measured at 37° C as described.

The stability of sevin, IPFC and 2-isopropoxyphenyl-*N*-methylcarbamate in aqueous solution was measured under the same experimental conditions as the rate of enzymic hydrolysis. Buffer solutions of the carbamates (0.15 and 0.8 m*M*) were incubated at 37° C; at suitable time intervals samples were withdrawn, and the amount of liberated phenols determined with the 4-aminoantipyrine reagents, as described.

RESULTS

Under given experimental conditions, human serum did not hydrolyse 2-isopropoxyphenyl-*N*-methylcarbamate; this carbamate was not hydrolyzed by fraction V either.

The rate of hydrolysis of 0.8 m*M* sevin and 0.8 m*M* IPFC in 12 sera is given in Fig. 1. There is a good linear correlation between the rates of hydro-

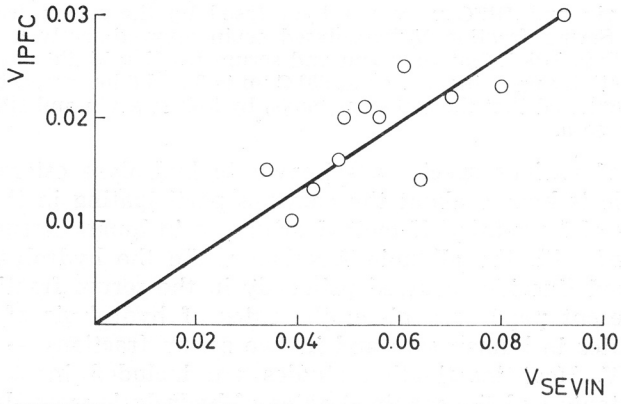

Fig. 1. Rates of hydrolysis of 1-naphthyl-*N*-methylcarbamate (v_{sevin}) and 3-isopropylphenyl-*N*-methylcarbamate (v_{IPFC}) in sera of different individuals. Rates are given in µmoles hydrolyzed substrate per min. per ml. of serum. The line is the calculated regression line.

lysis of the two compounds (correlation coefficient r = 0.81), and the calculated regression line passes through the origin. Sevin was hydrolysed faster than IPFC, the mean value for the rate of sevin hydrolysis being 3.1 times that for IPFC. The Michaelis constants (K_m) and the maximal rates of hydrolysis (*V*) were determined for each serum separately, by varying the substrate concentrations from 0.1 to 0.8 m*M*. The K_m and *V* values, obtained graphically from Lineweaver-Burk plots, are given in Table I. The Michaelis constants varied over a rather wide range, and no correlation was obtained either between K_m for sevin and K_m for IPFC, or between K_m and *V* for a given substrate. The maximal rates of hydrolysis also varied from one serum to the other, but for each serum V_{sevin} was higher than V_{IPFC}, the mean value of the ratio V_{sevin}/V_{IPFC} being 2.9.

Sevin, IPFC and 2-isopropoxyphenyl-*N*-methylcarbamate hydrolysed spontaneously under given experimental condition. The first order rate constants of hydrolysis were obtained graphically from Fig. 2 and are presented in Table I.

TABLE I

Michaelis Constants (K_m) and Maximal Rates (V) *of Hydrolysis of* N-*Methylcarbamates in Human Sera*

K_m is expressed in *M*, and *V* in μmoles hydrolyzed substrate per min. per ml. of serum; ± is the standard error of the mean. *k* (min^{-1}) is the rate constant of spontaneous hydrolysis of the substrates.

Substrate	$10^4 \cdot K_m$	$10^2 \cdot V$	$10^4 \cdot k$
1-naphthyl-*N*-methylcarbamate	17 ± 17	17 ± 11	11.5
3-isopropylphenyl-*N*-methylcarbamate	23 ± 21	5.7 ± 3.3	2.4
2-isopropoxyphenyl-*N*-methylcarbamate	not hydrolyzed		2.9

Fraction IV-1 did not hydrolyse sevin and IPFC. The hydrolysis of sevin and IPFC by fraction V was measured at 0.8 m*M* substrate. The rate of hydrolysis was neither a linear function of the enzyme concentration, nor was it linear with the time of hydrolysis. For this reason the Michaelis constants and *V* could not be evaluated. At a given concentration of fraction V and for a given time of reaction, the hydrolysis rate of sevin was 4.2 times greater than that of IPFC. This result was obtained as a mean of 15 experiments each done with a different enzyme concentration and a different time of hydrolysis.

Heat inactivation studies proved that native serum did not alter its hydrolytic activities up to 3 hr. at 53° C. The activity of fraction V was stable for 6 hr. at that temperature.

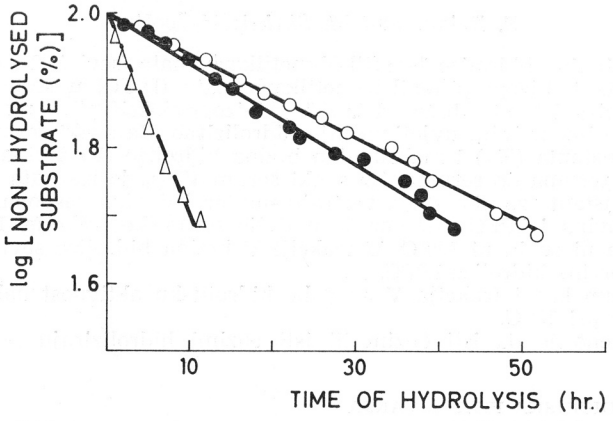

Fig. 2. Rates of spontaneous hydrolysis of 1-naphthyl-*N*-methylcarbamate (△), 2-isopropoxyphenyl-*N*-methylcarbamate (●), and 3-isopropylphenyl-*N*-methylcarbamate (○).

DISCUSSION AND CONCLUSION

Of the three monomethylcarbamates used as substrates, only two were hydrolyzed by serum, but all three compounds hydrolysed spontaneously. This lack of correlation between enzymic and spontaneous hydrolysis is analogous to the one observed in a series of derivatives of 4-nitrophenyl-*N*-methylcarbamates[4]. The hydrolytic activity of fraction V agrees well with findings of Casida and Augustinsson[2], that the albumin fraction of 8 species, including humans, hydrolyzed sevin. The good thermostability of this fraction agrees with the thermostability observed in native sera.

The high correlation between the rate of hydrolysis of sevin and IPFC, with the regression line passing through the origin, indicates that the same enzyme (or the same enzymes) hydrolyses both compounds in human sera. The experiments allowed no conclusion about the number of enzymes possibly involved. The Lineweaver-Burk plots were straight lines, what is to be expected if a single enzyme is involved, but could also be possible with two enzymes present[5]. The Michaelis constants varied from one serum to the other, with no correlation to either activity or *V*. A given serum with a high *V* value was not necessarily the one with a high rate of hydrolysis at constant substrate concentration, and *vice versa*. These results lead to the assumption that a rather complex enzyme system is operating in the hydrolytic process.

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IZVOD

Hidroliza nekih N-metilkarbamata u serumima ljudi

E. Reiner and M. Škrinjarić-Špoljar

Mjerena je brzina hidrolize 1-naftil-*N*-metilkarbamata (sevin), 2-izopropoksifenil-*N*-metilkarbamata i 3-izopropilfenil-*N*-metilkarbamata (IPFC) u serumima 12 ljudi. Sevin se hidrolizira 3,1 puta brže od IPFC, a 2-izopropoksifenil-*N*-metilkarbamat se pod danim eksperimentalnim uvjetima nije hidrolizirao. Za svaki serum određena je Michaelisova konstanta (K_m) i maksimalna brzina hidrolize (V) sevina i IPFC. I K_m i V variraju od seruma do seruma. Za svaki serum V_{sevin} je 2,9 puta veći od V_{IPFC}. Michaelisove konstante za oba spoja variraju unutar jednakih raspona.

Hidroliza sevina i IPFC mjerena je u dvije serumske frakcije. Frakcija IV—1 nije hidrolizirala ni sevin ni IPFC. U frakciji V brzina hidrolize sevina bila je oko 4 puta veća od brzine hidrolize IPFC.

Nativni serum kao i frakcija V ne gube hidrolitsku aktivnost nakon 3 odnosno 6 sati inkubacije pri 53° C.

Pretpostavljeno je da isti enzim ili isti enzimi hidroliziraju sevin i IPFC u serumima ljudi.

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