other hand, AC activity is also subject to allosteric activation[11] by cytosolic factors as well as by nucleotides like GTP[12-15]. If GTPases are inhibited, e.g. by choleratoxin, a highly activated and persisting state of AC activity will be elicited similar to that produced by Gpp(NH)p[16]. Moreover, in certain membrane preparations GTP and low concentrations of catecholamines can activate AC activity in an overadditive manner[14,17]. In some experiments we observed, like other authors[5], a decrease of high basal activity brought about by (±)propranolol (10^{-6} M) up to 50%. From these investigations, it might be speculated that in membrane preparations GTP is bound to a varying degree depending on the respective GTPase activity. When this enzyme activity is low, GTP and small amounts of catecholamines bound to the membranes might elicit highly preactivated states of AC activity which can be stimulated by Ipn, Gpp(NH)p and NaF respectively only to a minor degree. The possibility that other cytosolic factors[15], adherent to the cytoplasmic membrane, were also of influence on the variations of AC activity cannot be excluded.

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Inhibition of α-chymotrypsin by 2-halogeno-ethanols; comparison with 2-methyl-ethanols and urea

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Summary. 2-Halogeno- and 2-methyl-ethanols inhibit α-chymotrypsin in the order of their substituted groups: [1] tri- > di- > mono-, [2] Br- > Cl- > CH_3- > F-. The inhibition by the halogeno-ethanols is mediated differently from that by the methyl-ethanols, ethanol, and urea.

The introduction of a Cl-group instead of the methyl-group on 1-propanol (2-chloroethanol) enhances the ability to change protein conformation as reported by Tanford[1]. However, the effect derived from the change in the substitution number and species of halogeno- groups remains obscure. In this report, we present the effect of 2-halogeno-ethanols (2-fluoroethanol, 2,2,2-trifluoroethanol, 2-chloroethanol, 2,2,2-trichloroethanol, 2-bromoethanol, 2,2,2-tribromoethanol) on the hydrolytic activity of α-chymotrypsin on N-benzoyl-L-tyrosine-p-nitroanilide[2] and comparing them with 2-methyl-ethanols (1-propanol, 2-methyl-1-propanol, 2,2-dimethyl-1-propanol), ethanol, and urea.

Materials and methods. α-Chymotrypsin (bovine pancreas) and N-benzoyl-L-tyrosine-p-nitroanilide were obtained from Sigma, St. Louis, ethanol, 1-propanol, 2-methyl-1-propanol, 2,2-dimethyl-1-propanol, 2-fluoroethanol, 2-chloroethanol, 2,2,2-trichloroethanol, 2-bromoethanol from Wako, Osaka, and 2,2,2-trifluoroethanol and 2,2,2-tribromoethanol from E. Merck, Darmstadt.

Enzyme assay. The enzyme reaction was performed at 30 °C in 4 ml of a reaction mixture (pH 8.0) consisting of 1.9 ml of substrate-buffer (1 vol. of 1.25 mM N-benzoyl-L-tyrosine-p-nitroanilide in ethanol plus 4 vols of 0.1 M Tris-HCl, pH 8.0, 0.01 M $CaCl_2$), 2 ml of deionized water (a control) or 2 ml of aqueous solutions of urea or alcohols that are soluble in water in any proportion (A series: urea, ethanol, 1-propanol, 2-fluoroethanol, 2,2,2-trifluoroethanol, 2-chloroethanol, 2-bromoethanol) or 2 ml of 5 M ethanolic solutions of the alcohols, including some that are hardly soluble in pure water (B series: ethanol, 1-propanol, 2-methyl-1-propanol, 2,2-dimethyl-1-propanol, 2-fluoroethanol, 2,2,2-trifluoroethanol, 2-chloroethanol, 2,2,2-trichloroethanol, 2-bromoethanol, 2,2,2-tribromoethanol), and 0.1 ml of α-chymotrypsin in 0.001 N HCl (553 μg/ml). The p-nitroaniline liberated was determined spectrophotometrically at 400 nm.

Results and discussion. The p-nitroaniline liberated in 5 min by the α-chymotrypsin in the absence of urea and the alcohols (a control, 0.0825 μmoles) and in the presence of urea or the alcohols are given as function of the urea and alcohols concentration in figure 1.

In series A, urea in any concentration inhibited the enzyme. Ethanol in concentrations below 1 M had no effect on activity, and in higher concentrations inhibited the enzyme. These reagents caused no activation, but all the substituted ethanols in series A caused perceptible activation at low concentrations, whereas at high concentrations they inhibited the enzyme. Urea, which has no hydrophobic group, destabilizes native conformations by negative free energies of transfer from water to urea of hydrophobic groups[3-5] and polar groups[6]. Therefore, though it is not evident, it is possible that the different profiles in series A (urea and ethanol, inhibition; substituted ethanols, activation to inhibition) resulted from their different ability to invade the hydrophobic regions in the enzyme. The inhibition

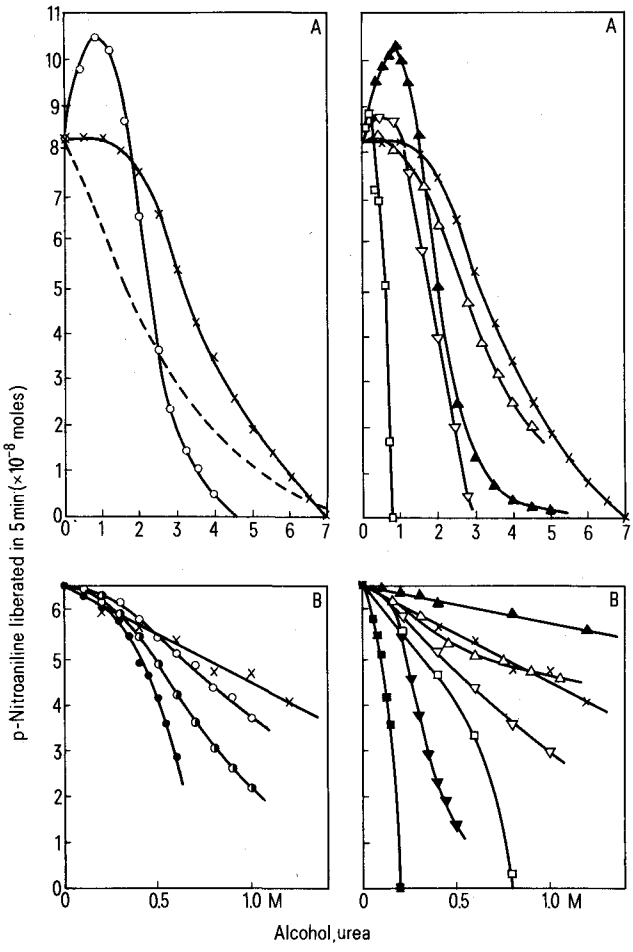

Fig. 1. Activities of α-chymotrypsin on N-benzoyl-L-tyrosine-p-nitroanilide in the presence of ethanol (×——×); 2-halogeno-ethanols: 2-fluoroethanol (△——△), 2,2,2-trifluoroethanol (▲——▲), 2-chloroethanol (▽——▽), 2,2,2-trichloroethanol (▼——▼), 2-bromoethanol (■——■); 2-methyl-ethanols: 1-propanol (○——○), 2-methyl-1-propanol (◑——◑), 2,2-dimethyl-1-propanol (●——●); and urea -----). Reaction was performed at 30 °C for 5 min in 4 ml of reaction mixture (pH 8.0): 1.9 ml of substrate-buffer, 2.0 ml of deionized water (a control) or 2.0 ml of aqueous solutions of the alcohols or urea (A), or 2.0 ml of 5 M ethanolic solutions of the alcohols (B), and 0.1 ml of the enzyme solution.

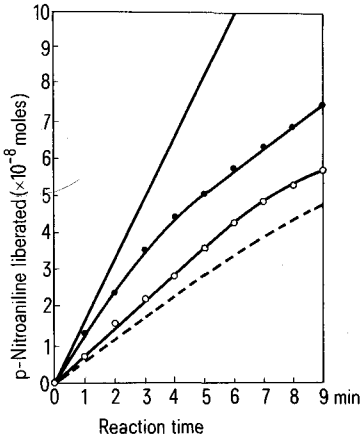

Fig. 2. Time-dependence of the activity of α-chymotrypsin in the presence of 2.5 M 2,2,2-trifluoroethanol (●——●), 2.0 M 1-propanol (○——○), 3.0 M urea (-----), and in the absence of any modifier (———), the conditions as for A in figure 1.

effectiveness of the alcohols among the substituted groups is ordered as Br- > Cl- > CH_3-, F_3- > F- (> ethanol).

In series B, all the alcohols inhibited the enzymes in the order of Br_3- > Cl_3- > $(CH_3)_3$- > Br- > $(CH_3)_2$- > Cl- > CH_3- > (> ethanol) > F- > F_3-. Because, considering the fluoro-ethanols and ethanol, reverse orders are obtained between A and B, the presence of ethanol apparently reduces the inhibition effect of the fluoro- groups.

From A and B, the inhibition effectiveness among the substituted groups is summarized as [1] tri- > di- > mono-, [2] Br- > Cl- > CH_3- > F-. These orders cannot be completely explained by van der Waals' volume[7] or the electro-negativity[8] of the groups.

The time-dependence of the activities with about 50% inhibition in figure 1A was examined. Figure 2 shows the profiles for 3.0 M urea, 2.5 M 1-propanol, and 2.0 M 2,2,2-trifluoroethanol. The reaction with urea and 1-propanol proceeded almost linearly up to 5 min and thereafter non-linearly, but with 2,2,2-trifluoroethanol, on the contrary, non-linearly up to 5 min and thereafter linearly.

The profiles with 0.5 M 2-bromoethanol and 2.0 M 2-chloroethanol were the same as that with the trifluoro-ethanol. The non-linear progress indicates the presence of slow change in the enzyme conformation; the change occurs more readily for the halogeno-ethanols than urea and 1-propanol. It is to be noted that the profile with 1.0 M 1-propanol (in the activation range) is almost the same as that with 2.5 M in the time below 5 min.

All this shows that the alcohols can be divided into 2 groups: halogeno-ethanols, methyl-ethanols, and ethanol according to their inhibition effect. The latter group rather resembles urea in its inhibiting behavior. X-ray studies[9,10] show that hydrophobic interaction among hydrophobic groups in the enzyme is important in maintaining the configuration of the active sites and the charge-relay system[10,11]; such hydrophobic groups are the same in quality as the methyl- group[13] and differ from the halogeno- groups[14] in their hydrophobic nature. Therefore, the halogeno-ethanols inhibited the enzyme probably through effective and rapid perturbation of the configuration and the methyl-ethanols, ethanol, and urea, on the contrary, through an extensive change in the native conformation rather than such local perturbation.

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