Active site structural change of α-chymotrypsin due to 2-halogeno-ethanols; comparison with ethanol, 1-propanol, and urea

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Summary. 2-Halogeno-ethanols change the active site structure of α-chymotrypsin more rapidly and effectively than ethanol, 1-propanol and urea, probably before producing an extensive conformation change.

The introduction of halogen-groups or methyl-groups into ethanol produces a decrease in its effective concentration for the inhibition of α-chymotrypsin *(EC 3.4.21.1)*, and in the lag in the appearance of the inhibition. Both the decreases are much more pronounced for the halogeno-groups than for the methyl-groups[1]. The methyl-ethanols rather resemble urea in the lag; inhibition by urea requires a relatively long lag. Such differences in inhibition ability between the halogeno-ethanols and the methyl-ethanols may be related to possible differences in their ability to change the structure of the active sites[1]. However, no comparative study has been reported about the structure-changing ability among them. 1-Bromo-*p*-nitroacetophenone alkylates the Met-192 of α-chymotrypsin (NAP-chymotrypsin) and forms a conformationally dependent charge-transfer complex with a vicinal Trp-residue[2]. The charge-transfer spectrum is useful as an indicator of change in the structure around the active sites. In this report, we have examined the effect of alcohols - 2,2,2-trifluoroethanol, 2-chloroethanol, 2-bromoethanol, 1-propanol, and ethanol - and urea on the spectrum in order to estimate their ability to change the structure around the active sites.

Methods. NAP-Chymotrypsin was prepared according to the alkylation procedures of α-chymotrypsin by *p*-nitrophenylbromo-1-amino-iso-butyrate[3]. Charge-transfer spectrum of NAP-chymotrypsin was obtained by the use of a 1 cm light-path cuvette with 2.6×10^{-6} M NAP-chymotrypsin dissolved in 0.001 N HCl and 9.5 vol.% ethanol as solvent[1] in a spectrophotometer (356 model, Hitachi). The spectrum with the solvent ethanol was the same as that without any ethanol, having a maximum at 347 nm and a minimum at 320 nm[2].

Results and discussion. The addition of urea or the alcohols caused a disappearance of the charge-transfer spectrum of NAP-chymotrypsin depending on their concentration and on time. Urea at a concentration of 8.0 M completely decreased the absorption at 347 nm after more than 10 min. Assuming that no surviving charge-transfer complex exists after more than 10 min with 8.0 M urea, we can conveniently estimate the surviving complex with the alcohols as well as urea by the equation,

$$(\Delta A_{sample} - \Delta A_0)/(\Delta A_{100} - \Delta A_0)\times 100,$$

where ΔA is $A_{347} - A_{320}$, ΔA_{100} is the value for the control (in the absence of urea and the alcohols), ΔA_0 is for 8.0 M urea after more than 10 min, ΔA_{sample} is for urea or the alcohols. The surviving complex thus obtained with 2,2,2-trifluoroethanol, 2-chloroethanol, 2-bromoethanol, 1-propanol, ethanol, and urea is given as a function of time in figure 1. The time required to break-up the complex in half, the half-life time of the complex, was obtained from figure 1, and is summarized in the table. The half-life time was less than 2 min for 2,2,2-trifluoroethanol above

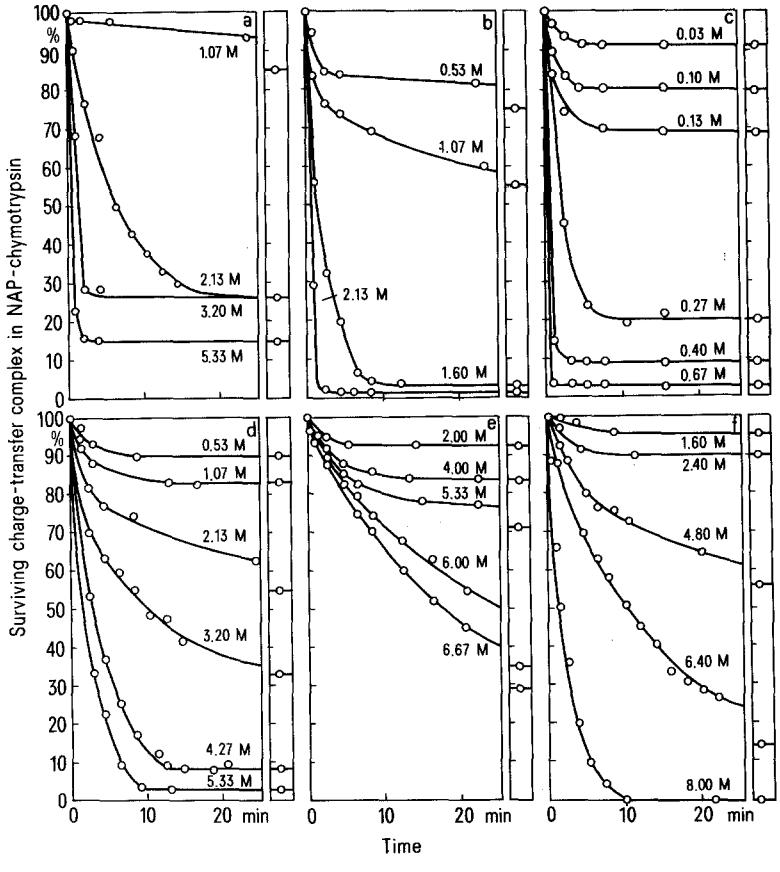

Fig. 1. Change in the surviving charge-transfer complex in NAP-chymotrypsin (α-chymotrypsin alkylated with 1-bromo-*p*-nitro-acetophenone) with time in the presence of various concentrations of alcohols (a, 2,2,2-trifluoroethanol; b, 2-chloroethanol; c, 2-bromoethanol; d, 1-propanol; e, ethanol) and urea (f).

Half-life time of charge-transfer complex in NAP-chymotrypsin in the presence of 2,2,2-trifluoroethanol, 2-chloroethanol, 2-bromoethanol, 1-propanol or ethanol

	Trifluoroethanol		Chloroethanol		Bromoethanol		Propanol		Ethanol	
Concentration of alcohol (M)	3.20	5.33	1.60	2.13	0.27	0.40	3.20	5.33	6.00	6.67
Half-life time (min)	1.5	<0.5	1.5	<0.5	1.5	<0.5	10.0	2.5	25.0	17.5

3.20 M, 2-chloroethanol above 1.60 M, and 2-bromoethanol above 0.27 M. However, the time was 10 min for 1-propanol at 3.20 M and 25 min for ethanol at 6.00 M. Figure 1 and table 1 show that the halogeno-ethanols changed the structure around the active sites more rapidly than ethanol and 1-propanol. Urea was similar to ethanol and 1-propanol, and changed it slowly as shown in figure 1. The surviving complex with the alcohols and urea at infinite time is given as a function of their concentration in figure 2. The concentration required to break-up the complex in half was 1.0 M for 2-chloroethanol, 0.2 M for 2-bromoethanol, 1.5 M for 2,2,2-trifluoroethanol, 2.5 M for 1-propanol, and 6.0 M for ethanol. The halogeno-ethanols, therefore, have an ability to cause a more effective and rapid change in the structure around the active sites of α-chymotrypsin than 1-propanol and ethanol; the latter group is similar to urea in that ability. We suppose that the difference in the ability of the alcohols and urea to inhibit α-chymotrypsin described in the introductory statement is due to differences in their ability to change the structure around its active sites. The similarity of urea to 1-propanol and ethanol seems to be contradictory to the differences in the denaturation process of a protein in urea and in organic solvents such as alcohols; denaturation in urea appears as a transition from the native to the denatured unfolded state[4-6], but putting a protein into a high concentration of organic solvents results in a structural ordering reaction through a partially unfolded state[4,6]. However, the apparent contradiction can be solved by the following possibility, which was proposed in the previous report[1]. The change in the structure around the active sites caused by 1-propanol and ethanol, as well as urea, is mediated through an extensive conformational change, and that caused by the halogeno-ethanols occurs possibly at first as a local structural change.

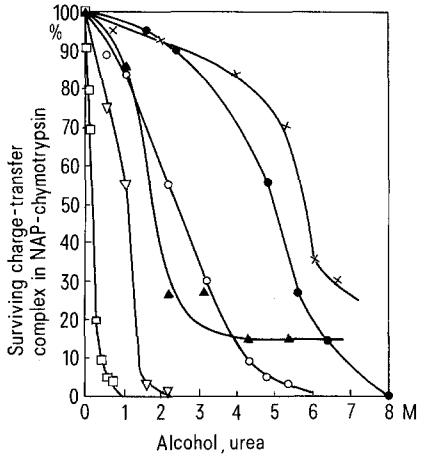

Fig. 2. Change in the surviving charge-transfer complex in NAP-chymotrypsin at infinite time with the concentration of alcohols (▲——▲, 2,2,2-trifluoroethanol; ▽——▽, 2-chloroethanol; □——□, 2-bromoethanol; ○——○, 1-propanol; ×——×, ethanol) and urea (●——●).

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Monoacylcadaverines as substrates for both monoamine oxidase and diamine oxidase; low rates of activity

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Summary. Monoacetylcadaverine and monopropionylcadaverine were found to be substrates for both rat liver monoamine oxidase and hog kidney diamine oxidase, but all the K_m-values for the oxidases were very high. The amines were common substrates for type A and type B monoamine oxidase.

Monoacetylcadaverine and monopropionylcadaverine were first identified in urine of schizophrenic patients[1]. Recently, Dolezalova et al.[2,3] also identified these acylcadaverines in blood of schizophrenic and normal subjects, and found that the levels of the monoacylcadaverines were higher in blood of schizophrenic patients than in that of the normal subjects. The physiological significance of these amines is not known.

The synthesis and degradation of the monoacylcadaverines have not been studied. Monoacetylcadaverine may be formed from cadaverine via acetylation, because it has been reported that putrescine is preferentially acetylated by rat brain tissue[4]. It is also to be expected that the monoacylcadaverines may be catabolized by monoamine oxidase (EC 1.4.3.4, MAO), since monoacetylputrescine is reported to be a substrate for MAO in the rat[5].

In this paper, we demonstrate that the monoacylcadaverines are substrates for MAO in vitro and further charac-