Designed inhibitors of horse liver alcohol dehydrogenase

Effect of active-site metal ion substitution

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3-(*p*-Butoxyphenyl)propionamide, -thioamide and -hydrazide and the formamide of *p*-butoxybenzylamine were tested as inhibitors of cadmium(II) and cobalt(II) active-site substituted alcohol dehydrogenase. The results agree with a direct coordination of these inhibitors except for the hydrazide to the active-site metal ion, in the enzyme-NADH-inhibitor complex. The hydrazide might be situated at some distance from the metal ion without a direct coordination bond.

Alcohol dehydrogenase *Enzyme inhibitor* *Metal ion substitution*

1. INTRODUCTION

A number of inhibitors of horse liver alcohol dehydrogenase (LADH) have been designed from the protein structure, prepared, tested in vitro and in vivo and found quite efficient [1,2]. This demonstrates the usefulness of knowledge of the receptor in order to design the structure of a ligand.

For this work, the active-site Zn^{2+} was considered to be one of the binding sites of the inhibitor. Since this enzyme with metal ion substitution at the active site is available [3], it was of interest to test some of the inhibitors with the cadmium(II)- and cobalt(II)-substituted enzymes, the results of which are presented here.

2. MATERIALS AND METHODS

The compounds **1**, **2**, **3** and **4** were prepared as described [1]. LADH (crystalline suspension) was purchased from Boehringer. For the tests the suspension was centrifuged at 4°C. The enzyme crystals were dissolved in 0.1 M phosphate buffer, pH 7.5. After dialysis against the same buffer, the solution was centrifuged to eliminate the denatured enzyme. The enzyme concentration and active-site content were determined according to [4]. The active site specifically substituted Co(II)- and Cd(II)-alcohol dehydrogenases were prepared as in [3,5]. Active-site concentrations of the Co(II)-enzymes were determined photometrically [6], that of the Cd(II)-enzymes fluorometrically [7]. NADH from Boehringer had an absorption ratio (260 nm/340 nm) below 2.5. Tetramethylurea (Aldrich), used to dissolve the inhibitors, was distilled twice under vacuum (12 mmHg). A number of tetramethylurea samples contained a strong inhibitor of LADH and were rejected. Acetaldehyde was prepared from paraldehyde (Fluka) by depolymerization with a minute amount of sulfuric acid and distilled 3 times. Enzymatic tests were run in 0.1 M phosphate buffer, pH 7.5, and inhibition constants determined according to Lineweaver-Burk and to Eadie-Scatchard.

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3. RESULTS AND DISCUSSION

Table 1 shows the K_i values for compounds **1–4** measured with alcohol dehydrogenases containing Cd, Zn and Co as catalytic metal ion. The data are compared with the K_m values measured for acetaldehyde. It has previously been shown that all inhibitors act competitively against acetaldehyde with the native zinc enzyme. This also holds true for the Co(II)- and Cd(II)-substituted derivatives. It is generally found that all K_i values are very similar for the Zn(II)- and Co(II)-enzymes, whereas the Cd(II)-enzyme shows markedly higher K_i values with respect to inhibitors **1–3**.

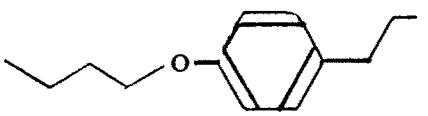
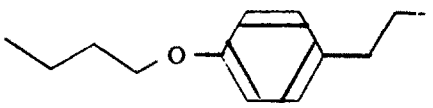
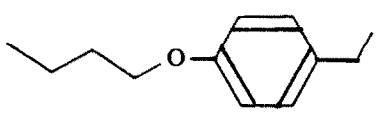
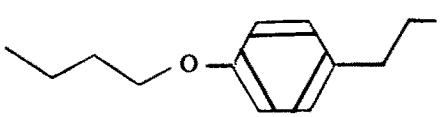
The hydrazide **4**, however, acts more weakly against all 3 enzyme species with nearly equal K_i values of about 2×10^{-4}. The K_i values found with all other combinations of metalloenzymes and inhibitors **1–3** are in the range 10^{-5}–10^{-8} and thus significantly lower than those obtained with the hydrazide **4**.

Two conclusions may be drawn from these observations: Firstly, the amide, thioamide and formamide inhibitors **1–3** seem to coordinate directly to the metal ion. This is shown by the higher inhibition constants displayed by the Cd(II)-enzyme as compared to the Zn(II) and Co(II) species. This would reflect the lower charge density of Cd(II) and parallels the weaker binding of and the lower rate of hydride transfer to the substrate *trans*-4-(*N*,*N*-dimethylamino)cinnamaldehyde (DACA) [8] measured with Cd(II)-enzyme as compared to Ni(II)-, Zn(II)- and Co(II)-enzyme.

In view of the higher donor strength of the thioamide compared to the amide, it is surprising to note that the former generally inhibits all 3 enzymes less strongly than the latter. However, steric crowding with the thioamide compared to that of

Table 1

Competitive inhibition constants of compounds **1–4** with respect to acetaldehyde and Michaelis constants of acetaldehyde with active-site metal ion-substituted horse liver alcohol dehydrogenase

K_i (μM)	Cd(II)	Zn(II)	Co(II)
 **1**	0.8	0.17	0.19
 **2**	44	4.9	5.7
 **3**	0.23	0.04	0.079
 **4**	230	190	160
CH_3-CHO	25000	200	90

the amide may explain the difference. The extraordinary high inhibitory action of the formamide is not easily explained from the electronic structure of the inhibitor molecule. It may be assumed that in addition to the coordinative bond between oxygen and metal ion, other interactions with protein groups may enhance the binding of this inhibitor. From graphic display studies, a hydrogen bond between the NH group of the formamide and Ser-48 seems feasible [1].

Secondly, the hydrazide **4** probably interacts in a manner different from that of the inhibitors bearing amide, thioamide or formamide moieties as functional groups. The hydrazide **4** was expected to be a good inhibitor in view of the high complexing ability of this class of compounds. Contrary to these expectations the inhibitory action is weak and notably independent of the kind of metal ion present at the catalytic site. Therefore, it can be questioned whether this interaction involves a direct coordinative bond at all, or whether the inhibitor is situated at some distance from the catalytic metal ion. It seems plausible that a hydrogen bond between the inhibitor molecule and the metal ion-bound water, inter alia, accounts for the fact that the inhibition constant is high and equal for all 3 metallo alcohol dehydrogenases tested. This type of bonding has been observed in the complex between Zn ADH-1,4,5,6-tetrahydronicotinamide adenine dinucleotide-2-methyl-2,4-pentanediol [9]. In addition, this type of positioning of the hydrazide inhibitor in the enzyme could also lead to less favorable accommodation of the bulky side chain.

Our data show a consistent picture of the behavior of the action of designed inhibitors with respect to all 3 metal ion-substituted alcohol dehydrogenases and thus indicate clear similarities in the active site. This conclusion, which has been independently derived from these kinetic experiments and others [10], is in full accordance with the results from X-ray structural studies of Co(II)- and Cd(II)-substituted LADH [11,12]. The small but significant differences observed in the kinetic experiments show the direct interaction between metal, substrate and inhibitor. Metal substitution proves to be a tool to investigate the dynamic behavior of the active site of metalloenzymes.

REFERENCES

[1] Freudenreich, C., Samama, J.P. and Biellmann, J.F. (1984) J. Am. Chem. Soc. 106, 3344–3353.
[2] Delmas, G., de Saint-Blanquat, G., Freudenreich, C. and Biellmann, J.F. (1983) Alcoholism Clin. Exp. Res. 7, 264–270.
[3] Maret, W., Andersson, I., Dietrich, H., Schneider-Bernlöhr, H., Einarsson, E. and Zeppezauer, M. (1979) Eur. J. Biochem. 98, 501–512.
[4] Theorell, H. and Yonetani, T. (1963) Biochem. Z. 338, 637–653.
[5] Andersson, I. (1980) Dissertation, Universität des Saarlandes, Saarbrücken, FRG.
[6] Einarsson, R., Widell, L. and Zeppezauer, M. (1976) Anal. Lett. 9, 815–823.
[7] Dietrich, H. (1980) Dissertation, Universität des Saarlandes, Saarbrücken, FRG.
[8] Dunn, M.F., Dietrich, H., MacGibbon, A.K.H., Koerber, S.C. and Zeppezauer, M. (1982) Biochemistry 21, 353–363.
[9] Cedergren-Zeppezauer, E., Samama, J.P. and Eklund, H. (1982) Biochemistry 21, 4895–4908.
[10] Koerber, S.C., MacGibbon, A.K.H., Dietrich, H., Zeppezauer, M. and Dunn, M.F. (1983) Biochemistry 22, 3424–3431.
[11] Schneider, G., Eklund, H., Cedergren-Zeppezauer, E. and Zeppezauer, M. (1983) Proc. Natl. Acad. Sci. USA 80, 5289–5293.
[12] Cedergren-Zeppezauer, E., Eklund, H. and Zeppezauer, M. (1985) Biochemistry, in press.