BINDING OF 5-CHLOROPYRIMIDIN-2-ONE TO HORSE LIVER ALCOHOL DEHYDROGENASE

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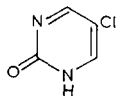
1. Introduction

5-Chloropyrimidin-2-one (I) belongs to a group of substances which exhibit a marked metaphase-arresting activity and for which the name metahalones has been proposed [1]. The mechanism of its action is not yet known but probably involves binding to specific target proteins.

We have found that 5-chloropyrimidin-2-one binds to horse liver alcohol dehydrogenase and that the binding is accompanied by a decrease in its fluorescence properties as well as by the specific inhibition of alcohol dehydrogenase. The experiments indicate that it binds to the rather unspecific hydrophobic binding site of alcohol dehydrogenase. In solvents of decreasing polarity the fluorescence intensity of 5-chloropyrimidin-2-one decreases markedly and the emission maximum shifts to higher wavelengths giving additional evidence for its binding to the hydrophobic binding site of the enzyme. Similar changes in the fluorescence spectrum might well occur when a metahalone binds to the target protein(s) and thereby causing metaphase arrest.



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2. Materials and methods

5-Chloropyrimidin-2-one was synthesized by method in [1,2].

Alcohol dehydrogenase from horse liver (EC 1.1.1.1), EE isoenzyme, was isolated and characterized as in [3]. Purity of the enzyme was ~85%. Enzyme concentrations are quoted with respect to the molar concentration of subunits.

Coenzymes NAD and NADH (grade II) were supplied by Boehringer, Mannheim; AMP was the product of Reanal, Budapest. Solvents used were of spectroscopic grade. The other chemicals were of analytical purity.

Kinetic measurements were performed with the spectrophotometer Cary 118 in Na-phosphate buffer pH 7.0 (ionic strength is 0.1) at 25°C. Initial reaction velocity was calculated from the rate of production or consumption of NADH.

The uncorrected fluorescence spectra were measured in an Aminco-Bowman spectrofluorometer, fluorescence polarization data were obtained on the same apparatus equipped with Glan prism polarizers.

3. Results and discussion

5-Chloropyrimidin-2-one inhibits the enzymic activity of alcohol dehydrogenase. It binds to the enzyme as a competitive inhibitor expelling the coenzyme NAD or NADH (see table 1). The values of the respective inhibition constants suggest a relatively high affinity to alcohol dehydrogenase comparable to its metaphase arresting effect (a significant metaphase arrest has been observed at 0.5 mM). As 5-chloropyrimidin-2-one competes with the coenzyme, it seems probable that it binds in the coenzyme binding domain of alcohol dehydrogenase. In order to determine the position of its binding site more exactly we performed kinetic inhib-

Table 1
Analysis of inhibition of alcohol dehydrogenase by 5-chloropyrimidin-2-one at 0, 0.5, 1.0, 1.5 mM and enzyme at 0.1 μM

Range (mM)	Additions (mM)	Observed behaviour	Av. K_i (mM)
NAD (0.005–0.15)	Ethanol (10)	Competitive inhibition[a]	0.55
NADH (0.005–0.10)	Acetaldehyde (5)	Competitive inhibition[a]	0.4
o-Phenantroline (0–0.10)	Ethanol (10) and NAD (0.5)	Non-exclusive binding[b]	–
AMP (0–0.35)	Ethanol (10) and NAD (0.05)	Exclusive binding[b]	–

[a] Data plotted according to Lineweaver-Burk and Hanes [6]
[b] Data plotted according to Yonetani-Theorell [6]

Brackets in the table indicate concentrations of the other components. Initial reaction rates were measured in sodium phosphate buffer (pH 7)

itors of alcohol dehydrogenase (*o*-phenantroline and AMP). *o*-Phenantroline binds to the enzyme approximately in the same area where the nicotinamide ring of the coenzyme is bound (close to the substrate binding site and the catalytic zinc of alcohol dehydrogenase [4]). AMP binds as does the AMP moiety of the coenzyme [4]. 5-Chloropyrimidin-2-one is able to bind to the enzyme simultaneously with *o*-phenantroline (see table 1). However, it expels AMP from its binding site as shown by the same slope of the Yonetani-Theorell plots suggesting the exclusive binding (see table 1). It is probable that its binds to the rather unspecific hydrophobic adenine-binding site of alcohol dehydrogenase. This binding site is fairly open and known to bind a number of coenzyme analogues, where other rings replace adenine (e.g., salicylate or anilinonaphtalene sulphonate [4,5]).

The binding of the AMP moiety of coenzyme is similar in all NAD-dependent dehydrogenases. Therefore, an interaction of 5-chloropyrimidin-2-one with other similar enzymes could be expected. However, preliminary results with lactate and glutamate dehydrogenases showed small effect on the respective enzymic activities at 2 mM. It can be concluded that the adenine binding site of alcohol dehydrogenase has a relatively specific ability to interact with 5-chloropyrimidin-2-one.

The metahalones are strongly fluorescent in water and other polar solvents. Excitation maximum for 5-chloropyrimidin-2-one in water is ~320 nm, fluorescence emission is maximal at ~370 nm. Fig.1 illustrates the influence of decreasing polarity of the solvent; fluorescence intensity decreases markedly and emission maximum shifts to higher wavelengths. Interaction of 5-chloropyrimidin-2-one with alcohol dehydrogenase is accompanied by a significant fluorescence decrease (see fig.2). This phenomenon confirms the hydrophobic character of the respective binding site in the

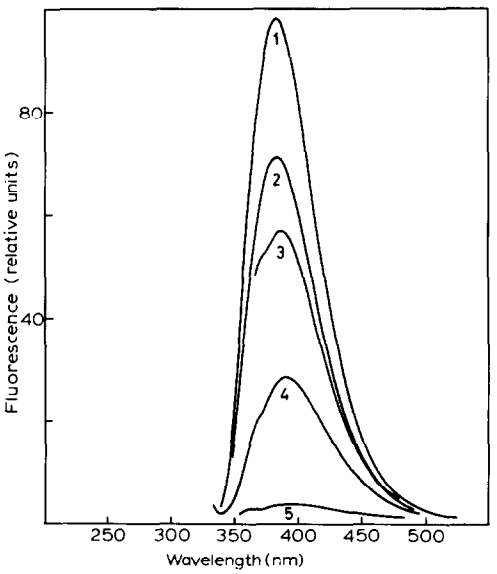

Fig.1. Fluorescence emission spectra of 5-chloropyrimidin-2-one in various solvents at 10 μM. Spectra were measured at 25°C at an excitation wavelength of 320 nm. The solvents were: water (curve 1); water/methanol (1:1) curve 2); methanol (curve 3); ethanol (curve 4); dioxane (curve 5).

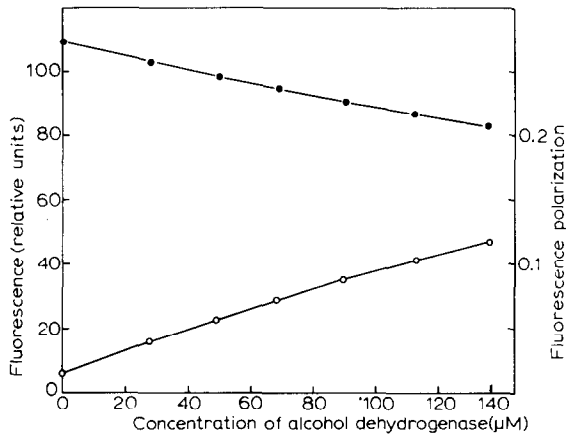

Fig.2. Changes in fluorescence (•) and fluorescence polarization (○) 5-chloropyrimidin-2-one (10 μM) in the presence of increasing concentration of alcohol dehydrogenase. The wavelengths for excitation and emission were 320 and 370 nm. Measurements were carried out in sodium phosphate buffer pH 7.0 (ionic strength 0.05) at 25°C.

enzyme molecule. However, fluorescence polarization increases upon binding (see fig.2) which is in accordance with the decreased mobility of the bound fluorophore. A quantitative evaluation of the titration curves shown in fig.2 was impossible for technical reasons (the enzyme concentration could not exceed 150 μM). The specificity of the observed changes in fluorescence and fluorescence polarization in the presence of alcohol dehydrogenase was confirmed by the finding that additions of lactate dehydrogenase, albumin or DNA in comparable concentrations exhibited practically no effect on the fluorescence of 5-chloropyrimidin-2-one.

It is very probable that the binding site for 5-chloropyrimidin-2-one and other metahalones to the specific target proteins (e.g., microtubular protein) would be more hydrophobic than water and that the mobilities of the bound substance would be restricted. Therefore, changes in fluorescence properties of metahalones similar to those observed in the presence of alcohol dehydrogenase are to be expected. Investigation of such changes with cellular proteins could provide a suitable method for detailed characterization of the biological activity of metahalones.

References

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