Inhibition Studies of Dehydrogenases by Structural Analogues of NAD$^+$

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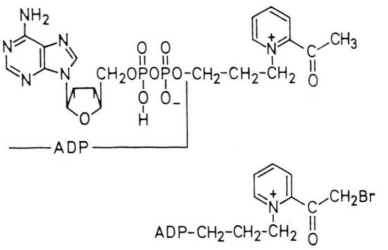
The coenzyme anologues [3-(2-acetylpyridinio)-propyl]-adenosine pyrophosphate and [3-(2-bromoacetylpyridinio)-propyl]-adenosine pyrophoshate were synthesized and used for inhibition studies. While the first compound reacts as competitive inhibitor against NAD$^+$, the latter is able to form covalently bound derivatives of several NAD$^+$-dependent dehydrogenases, giving informations about amino acid side chains participating in the binding of the functional coenzyme part.

[3-(2-acetylpyridinio)-propyl]-adenosine pyrophosphate exhibits strong hypochromicity between adenine and 2-acetylpyridine ring. Cleavage of the pyrophosphate bridge increases the absorption at 261 nm up to 22% and indicates, that the stacked configuration of the molecule is preferred in aqueous solution.

In enzymatic assays with YADH and GAPDH [3-(2-acetylpyridinio)-propyl]-adenosine pyrophosphate acts as a competitive inhibitor against NAD$^+$. The inhibition constants are: $K_I = 1.7 \cdot 10^{-2}$ M (YADH) and $4.3 \cdot 10^{-4}$ M (GAPDH) and show, that the coenzyme analogue has little affinity to the coenzyme binding sites. Difference spectra of the coenzyme-enzyme mixtures and the isolated components do not show any spectral changes due to the formation of the binary complex in the case of YADH and only small effects with GAPDH. From this results it can be concluded, that the incorporation of the 2-acetylpyridinio-propyl residue into the enzymes is sterically hindered. This is also indicated by the fact, that YADH shows smaller inhibition constants with adenosine monophosphate and adenosine diphosphate [1].

We obtained [3-(2-bromoacetylpyridinio)-propyl]-adenosine pyrophosphate by bromination of the acetyl group of the coenzyme analogue. The brominated compound is unstable in aqueous solution: At pH 6.5 the halftime is 120 min. At pH 7.5 the time is 20 min and at pH 8.5 it is 3 min.



[3-(2-Bromoacetylpyridinio)-propyl]-adenosine pyrophosphate inactivates ADH from yeast and GAPDH from rabbit muscle. ADH from horse liver and LDH do not react with the compound. $6 \cdot 10^{-5}$ M solution of GAPDH is inactivated at pH 6.5 in presence of $1 \cdot 10^{-4}$ M inactivator in 3 min to half activity, in presence of $1 \cdot 10^{-3}$ M inactivator the halftime is less then 30 sec. YADH is quickly inactivated either. $6 \cdot 10^{-5}$ M YADH is inactivated by $2 \cdot 10^{-4}$ M inactivator concentration at pH 6.5 within 8 min to half activity. If the inactivator concentration is $1 \cdot 10^{-3}$ M the time is less then 100 sec. In presence of NAD$^+$ or NADH the inactivation rate is decreased. Following the method of Gold and Fahrney [2], the apparent dissociation constant of the inactivator-YADH complex is $K_I = 2 \cdot 10^{-2}$ M; the rate constant is $k_2 = 10\ \text{min}^{-1}$. For GADPH the constants are: $K_I = 9 \cdot 10^{-4}$ M, $k_2 = 2\ \text{min}^{-1}$. In order to get informations about amino acid side chains, envolved in the inactivation reaction of YADH or GAPDH, we investigated the pH dependency of the inactivation rate. The curve showed an increased slope between pH 6 and 7 for YADH. The fast hydrolysis of the coenzyme analogue above pH 7.5 did not allow determination of the pK of the participating group without doubt. Up to pH 7.5 the curve of the pH dependency of the inactivation rate is similar to that with [3-(3-bromoacetylpyridinio)-propyl]-adenosine pyrophosphate [3]. This inactivator modified a histidine residue, which according to our results is near the pyridinio nitrogen. In the case of GAPDH [3-(2-bromoacetylpyridinio)-propyl]-adenosine pyrophosphate does not show any change in the inactivation rate between pH 5 and 7.5. After total in-

Abbrevations: YADH, alcohol dehydrogenase from yeast and LADH, alcohol dehydrogenase from horse liver = alcohol: NAD-oxidoreductase (EC 1.1.1.1); GAPDH, glyceraldehydephosphate dehydrogenase from rabbit muscle = D-glyceraldehyd-3-phosphate: NAD-oxidoreductase (phosphorylating) (EC 1.2.1.12); LDH, lactate dehydrogenase = L-lactate: NAD-oxidoreductase (EC 1.1.1.27).

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activation the number of SH-groups, measured by the method of Ellman [4], is reduced by 0.95 moles per mole subunit, suggesting, that a cystein residue is involved in the inactivation reaction.

We are not able to state definitly the position of this SH-group. The high inactivation rate, which is diminished in presence of NAD^+ or NADH suggests, that the reactive cysteine residue of the active site is envolved in the inactivation reaction. But the high dissociation constant of the inactivator-GAPDH complex does not agree with this assumption, it shows, that there is little or no affinity between the functional coenzyme binding site and the 3-bromoacetylpyridinio-propyl residue.

2-Acetyl-1-(hydroxypropyl)-pyridinio bromide was prepared by heating a mixture of 6.06 g 2-acetylpyridine and 6.96 g 3-bromopropanol-(1) for 30 h on a steam bath. The purification of the product and its phosphorylation was performed according to methods described previously [5]. Yield 2.5 g 3-(2-acetylpyridinio)-propyl phosphate; m.p. 138 – 140 °C; $UV_{max}(H_2O)$ 271 nm ($\varepsilon = 5.600$).

$C_{10}H_{14}NO_5P \cdot HCl$ (mol.wt. 295.7)
Calcd: 40.62 C 5.11 H 4.74 N 11.99 Cl 10.48 P,
Found: 40.76 C 4.99 H 4.82 N 12.19 Cl 10.60 P.

980 mg [3-(2-acetylpyridinio)-propyl]-adenosine pyrophosphate were obtained from 900 mg 3-(2-acetylpyridinio)-propyl phosphate and 1.8 g adenosine-5′-phosphoromorpholidate [6] and following purification on Dowex 1×8 ion exchange resin [7]; UV_{max} (H_2O) 261 nm ($\varepsilon = 17.200$).

$C_{20}H_{26}N_6O_{11}P_2 \cdot 0.66$ acetone (mol.wt. 627,1)
Calcd: 42.13 C 4.82 H 13.40 N 9.88 P,
Found: 42.09 C 4.94 H 13.27 N 9.82 P.

[3-(2-Bromacetylpyridinio)-propyl]-adenosine pyrophosphate was prepared as described for an isomeric compound [7]. The methods used in this work are corresponding with those previously reported [7].

GAPDH 130 U/mg was prepared from rabbit muscle [8], YADH, LADH, LDH, NAD^+ and NADH were purchased from Boehringer & Soehne, Mannheim.

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