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Kinetics and docking studies on the effect of chemical modification of NADH for redox reactions with dehydrogenases

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ABSTRACT

Cofactor analogs promise important applications in biosynthesis. The effect of chemical modification on the reactivity of NADH for redox reactions catalyzed by dehydrogenases was examined in this work. Compared with the native NADH, kinetics and molecular docking studies with 8-(6-aminohexyl)-amino-NADH showed that its binding with alcohol dehydrogenase (ADH) was not much affected or even enhanced by a factor of 4.9-fold with lactate dehydrogenase (LDH), but complicated the binding of substrates to the enzymes. For ADH, the Michaelis constant for acetaldehyde decreased from 0.47 to 0.048 mM, while that of sodium pyruvate with LDH increased to 0.81 from 0.18 mM. On the other hand, the modified coenzyme showed a 19.3-fold decrease in turnover number (k_{cat}) with ADH, while a slight increase with LDH. Molecular docking analysis showed that the hexanediamine arm on the modified coenzyme generated an extra hydrogen bond at the active site of ADH, as well as additional hydrophobic interactions with both ADH and LDH. It appeared that the apparently decreased reactivity of modified cofactor with ADH was caused mainly by the enhanced stability of ternary coenzyme–enzyme–substrate complex, while in the case of LDH, the reduced substrate binding as a result of the chemical modification of NADH led to a slight increase in the overall reaction reactivity.

1. Introduction

Site-directed mutagenesis of the key amino acids in active sites of coenzyme-dependent oxidoreductases has been proven effectively in manipulating enzyme activity and specificity by affecting the binding of coenzyme to corresponding enzymes [1–3]. Generally speaking, enzyme–coenzyme binding is one of the most critical steps in controlling the overall efficiency of redox transformation reactions [4]. In this regard, changing the structure of coenzyme NAD(H) through chemical modification will potentially provide an alternative strategy to genetic modification of enzyme since the complexation of enzyme–coenzyme and subsequent steps in reaction mechanism might be changed accordingly [4]. Within 1970s to 1980s, a great variety of chemical reaction routes were developed for coenzyme modification, and related works were comprehensively reviewed by Bückmann and Carrea [5]. One of the main objectives of coenzyme modification was to facilitate affinity purification of coenzyme-dependent oxidoreductases [6–10], while little attention was paid to retaining activity of the modified coenzyme. The other important application of modified cofactor was aiming at sustainable enzymatic biosynthesis. Cofactor-dependent enzymatic reactions have a wide spectrum of applications in organic synthesis especially chiral materials [11–14]. For continuous production in membrane reactors [5,15,16] or with semi-permeable microcapsules containing multienzymes and coenzyme [17,18], chemically modified cofactors were usually required. For this application, coenzyme derivatives with good activity have been a considerably challenging subject [19–22].

It has been demonstrated that both position of modification (N-1, N^6 or C-8 of the adenine ring) and property of substitution groups (charge, hydrophobicity or hydrophilicity) have profound effects on the activity of coenzyme derivatives [5]. Hendle et al. [4] prepared a series of coenzyme derivatives by functionalizing the adenine moiety with small molecules possessing different charges such as propane sultone and 2,3-epoxypropyl-trimethyl-ammoninum chloride. The catalytic activity measured with lactate dehydrogenase isoenzyme H_4 showed that N^6-postion substituted NAD^+ derivatives were 25- to 250-fold more active than N-1 derivatives, and the positive charged substitution was more active than negative charged one, yet all derivatives were less active compared with the native NAD^+. Bückmann and Carrea [5] sumarrized the activity of functionalized coenzyme derivativs with repect to several dehydrogenases, in most cases, a decrease of V_{max} is accomponied by an increase of K_m.

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Mechanism of coenzyme-dependent oxidoreductases catalyzed reaction is complicated that generally involves the binding of coenzyme to enzyme, the formation of coenzyme–enzyme–substrate ternary, the hydride transfers, as well as the dissociation of product and oxidized or reduced coenzyme. Analysis on the apparent Michaelis–Menten constants V_{max} and K_m of enzymes with native and modified coenzyme therefore usually could not elucidate the effect of modification of coenzyme on the individual step in the reaction mechanism [23,24]. The development in modern computational simulation tools provides opportunities to study the molecular mechanism interpreting relationship between the activity and structure of coenzyme derivatives. Hendle et al. [4] used AMBER, a docking tool based on energy minimization theory, to study molecular mechanics affecting the recognition of modified coenzyme by lactate dehydrogenase. It was found that though N^6-(3-sulfopropy1)-NAD (6SP-NAD^+) had the lowest interaction energy with lactate dehydrogenase and one third of the K_m of native NAD^+, but its turn over number k_{cat} was only one fourth of the value of NAD^+. AMBER program was also applied to studying the relationship between activity of NAD^+ derivatives modified in the nicotinamide group and their geometries in ternary complex with alcohol dehydrogenase [23,24]. The 'out-of-plane' rotation of the side chain of the pyridinium ring in geometry of NAD^+ derivatives in dehydrogenase was considered decisive for its activity. In all above studies, the effect of chemical modification of coenzyme on its activity was only analyzed from the point of recognization and binding of coenzyme to the active sites in respective dehydrogenases. The lack of comprehensive analysis on the effect of structural change in coenzyme on substrate binding and other subsequent steps in reaction mechanism makes the explanation of effect resulting from coenzyme modification still rather speculative.

From above considerations, full kinetic studies incorporating with molecular docking analysis by taking exact reaction mechanism into account will provide further insight into relationship between the structure of coenzyme derivatives and its activity with respective dehydrogenase. Therefore, in the present study 8-(6-aminohexyl)-amino-NADH (C^8-HAD-NADH), a coenzyme derivative suitable for further macromolecularization or linking to solid matrix [5,7], was prepared by substituting the C-8 of adenine of NADH with 1,6-hexanediamine. Alcohol dehydrogenase and lactate dehydrogenase, both have wide range of applications in biosynthesis were selected as model dehydrogenases to comprehensively study the effect of modification of coenzyme on its catalytic activity by kinetics study and molecular docking study at the same time. Auto Docking based on Larmarckian Genetic Algorithm (LGA) that has been successfully used to study the binding of small ligand to macromolecular was applied for the molecular docking study [25–28].

2. Material and methods

2.1. Materials

Alcohol dehydrogenase (ADH) from yeast (EC: 1.1.1.1, lyophilized powder with a protein content of 90%), cofactor NAD^+ and NADH were purchased from Sigma Chemical Co. (St. Louis, USA). Lactate dehydrogenase (LDH) from *Staphylococcus* sp. (EC: 1.1.1.28, lyophilized powder) was obtained from Amano Enzyme Inc. (Nagoya, Japan). Sodium pyruvate was purchased from Acros Organics (Gell, Belgium). DEAE-Sepharose FF was purchased from GE Healthcare (Uppsala, Sweden). All other reagents including alcohol, acetaldehyde, sodium carbonate, sodium bicarbonate, liquid bromine, dimethyl sulfoxide (DMSO), 1,6-hexanediamine, and carbon tetrachloride were all of analytical grade.

2.2. Synthesis of 8-(6-aminohexyl)-amino-NADH

8-(6-Aminohexyl)-amino-NADH (C^8-HAD-NADH) were synthesized following a procedure modified from that reported by Lee et al. [7]. The synthesis reaction was started from NAD^+ due to the fact that NAD^+ is more stable in acid aqueous solution but labile in base solution, while NADH is subject to serious decomposition in acid [29]. Typically, NAD^+ (100 mg) was dissolved in 5 ml sodium acetate buffer (pH 4.0, 0.1 M) to which liquid bromine was added in four portions (25 μl each) at intervals of 30 min under magnetic stirring at room temperature. The unreacted bromine was removed by repeated extraction with carbon tetrachloride until neither phase changed color. Br-NADH has been found to be more thermally stable than Br-NAD^+ [30], therefore Br-NAD^+ was enzymatically reduced to Br-NADH by ADH prior to the next displacement reaction. The reduction of Br-NAD^+ was verified by the increase in absorbance at 340 nm using USB2000 UV–Vis spectrophotometer (Ocean Optics Inc., Dunedin, FL USA). Once the reduction was complete, the ratio of the absorbance at 340–260 nm was approximately 0.33 [7]. Then the mixture was dried using a rotary evaporator.

To displace the bromine at the C-8 position of adenine with 1,6-hexanediamine, 100 mg Br-NADH was dissolved in a 10 ml DMSO solvent containing 5 g 1,6-dianminohexane. The reaction was carried out at 70 °C, and the completion of the displacement of Br with 1,6-dianminohexane was verified by the shifting of wavelength of maximum absorbance from 263 nm to 280 nm, usually the reaction lasted for 4 h [7]. To purify the C^8-HAD-NADH, the 20-fold diluted reaction mixture was loaded to DEAE-Sepharose FF column (i.d., 1.0 cm × 13 cm) that was pre-equilibrated with 1 M sodium carbonate and water, 50 mM sodium carbonate buffer (pH 9.3) was used to elute the product from the column. Fractions with maximal absorbance at both 280 and 340 nm were pooled and dried on vacuum rotary evaporator at 40 °C, from which C^8-HAD-NADH was obtained as white powder. The overall yield of C^8-HAD-NADH was estimated about 60% based on the initial NAD^+ amount. The chemical modification on the C-8 position of NADH was confirmed by ^{1}H NMR and MALDI-TOF MS analysis of native NADH and C^8-HAD-NADH. NADH, ^{1}H NMR (δ ppm): 8.53 (s, C_2-adenine proton), 8.29 (s, C_8-adenine proton), MS: 664.4 (Mt 664.4); C^8-HAD-NADH, ^{1}H NMR (δ ppm): 8.04 (s, C_2-adenine proton), 1.34-1.69 (m, the hexyl protons); MS: 780.5 (Mt 780.5).

2.3. Kinetic characterization of native and modified NADH

The activity of native and modified NADH was evaluated by two enzymatic reduction reactions, which are ADH catalyzed reduction of acetaldehyde to ethanol and LDH catalyzed reduction of sodium pyruvate to lactic acid. Both reactions were conducted at 25 °C in 200 mM Tris–HCl buffer (pH 8.0), their reaction rates were acquired by measuring the decrease in absorbance at 340 nm ($\varepsilon^{NADH} = 6.22\ mM^{-1}\ cm^{-1}$). For kinetic studies, initial velocities were measured with substrates concentrations being varied at each of four coenzyme concentrations as follows: 0.04, 0.06, 0.08, and 0.1 mM. To determine the kinetic data of ADH with NADH or C^8-HAD-NADH as coenzyme, the acetaldehyde concentration ranged from 0.1 to 0.25 mM or 0.04 to 0.1 mM, respectively. To determine the kinetic data of LDH, the sodium pyruvate concentration ranged from 0.1 to 0.4 mM or 1 to 4 mM by using NADH or C^8-HAD-NADH as coenzyme, respectively.

2.4. Modeling of coenzyme binding to enzyme by AutoDock

The structures of ADH and LDH (PDB codes 2HCY and 1J49, respectively) were directly taken from the Protein Data Bank (PDB), http://www.rcsb.org/pdb. The crystal structure of ADH