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Immobilization of arginase and its application in an enzymatic chromatographic column: Thermodynamic studies of nor-NOHA/arginase binding and role of the reactive histidine residue

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Abstract

A biochromatographic approach is developed to measure for the first time changes in enthalpy, heat capacity change and protonation for the binding of nor-NOHA to arginase in a wide temperature range. For this, the arginase enzyme was immobilized on a chromatographic support. It was established that this novel arginase column was stable during an extended period of time. The affinity of nor-NOHA to arginase is high and changes slightly with the pH, because the number of protons linked to binding is low. The determination of the enthalpy change at different pH values suggested that the protonated group in the nor-NOHA–arginase complex exhibits a heat protonation of approximately −33 kJ/mol. This value agrees with the protonation of an imidazole group. Our result confirmed that active-site residue Hist 141 is protonated as imidazolium cation. Hist 141 can function as a general acid to protonate the leaving amino group of L-ornithine during catalysis. The thermodynamic data showed that nor-NOHA–arginase binding, for low temperature (<15 °C), is enthalpically unfavourable and being dominated by a positive entropy change. This result suggests that dehydration at the binding interface and charge–charge interactions contribute to the nor-NOHA–arginase complex formation. The temperature dependence of the free energy of binding is weak because of the enthalpy–entropy compensation caused by a large heat capacity change, $\Delta C_p = -2.43$ kJ/mol/K, of arginase. Above 15 °C, the thermodynamic data ΔH and ΔS became negative due to van der Waals interactions and hydrogen bonding which are engaged at the complex interface confirming strong enzyme–inhibitor hydrogen bond networks. As well, by the use of these thermodynamic data and known correlations it was clearly demonstrated that the binding of nor-NOHA to arginase produces slight conformational changes in the vicinity of the active site. Our work indicated that our biochromatographic approach could soon become very attractive for studying other enzyme–ligand binding.

Keywords: Enzyme; HPLC; Ligand

1. Introduction

Arginase, a 105 kDa homotrimer containing a binuclear manganese cluster in each protomer, catalyzes the hydrolysis of L-arginine to form L-ornithine and urea through a metal-activated hydroxide mechanism [1–2]. The binuclear manganese cluster is located at the base of a 15-Å deep active-site cleft in each monomer. The metal ion that is more deeply situated in the active site cleft (designed Mn_A^{2+}) is coordinated by four amino acids and a solvent molecule, with square pyramidal geometry [3–6]. The second metal ion Mn_B^{2+} is coordinated by four amino acids and the bridging solvent molecule in distorted octahedral fashion. The Mn_A^{2+}–Mn_B^{2+} separation is 3.3 Å. All metal ligands except for aspartate 128 (Asp 128) make hydrogen-bond interactions with other protein residues, and these interactions contribute to the stability of the metal binding site [5]. The arginase structure is the first atomic resolution structure of a functional metalloenzyme that has a specific catalytic site and

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physiological requirement for two Mn^{2+} ions. The catalytic metal requirement is rooted in the preferred geometry of manganese coordination, which properly orients the metal-bridging solvent molecule for catalysis [3]. As a metal-bridging solvent molecule must satisfy the coordination preferences of two manganese ions simultaneously, its position, and therefore its optimal catalytic activity, would be highly sensitive to the substitution of one or both Mn^{2+} ions. Coordination of a catalytic group to two metals rather than one may enhance the dependence of optimal catalytic activity on proper metal selectivity. Only two other polar residues are found in the immediate active site: glutamate 277 (Glu 277) and histidine 141 (Hist 141). Glu 277 is located deep in the active-site cleft 4.5 Å away from $Mn_A{}^{2+}$. A $\approx 20^{\circ}$ conformational change about side chain torsion angle χ^2 to orient Glu 277 would yield an ideal salt link with the substrate guanidinium group. Moreover, this interaction would position the electrophilic guanidinium carbon of the substrate directly over the metal-bridging solvent molecule, which is likely to be nucleophilic hydroxide ion in the active catalyst. It is unlikely that the deprotonated substrate guanidinium group binds directly to the metal(s) because of its high pK_a of 13.5 [7]. The side chain of His 141 is located about half-way out of the active-site cleft. Interestingly, arginase with asparagine (Asn or N with amino acid nomenclature) substituted for histidine at position 141 (Hist 141 → Asn arginase) retains roughly 10% residual activity compared with the wild-type enzyme [7]. Given its location 4.2 Å away from the metal-bridging solvent molecule, it is possible that Hist 141 is a proton shuttle in catalysis, mediating proton transfer to and from bulk solvent. Direct proton transfer with bulk solvent may be operative in the absence of Hist 141, which could account for the significant residual catalytic activity of Hist-141 → Asn arginase. A proton shuttle function for Hist 141 of arginase would be analogous for Hist 64 in the zinc metalloenzyme carbonic anhydrase II [8]. On opposite sides of the active-site lip, charged residues are found which may contribute to the exquisite specificity of substrate recognition [9]. Two isoenzymes have been identified in mammals: arginase I catalyses the final cytosolic step of the urea cycle in liver, and arginase II is a mitochondrial enzyme that functions in L-arginine homeostasis in non-hepatic tissues. For example, arginase I may regulate substrate L-arginine bioavailability to NO synthase in the immune response. Macrophage arginase I and NO synthase are reciprocally regulated at the level of transcription: NO synthase is induced by T-helper type 1 (TH1) cytokines, and arginase I is induced by T-helper type 2 (TH2) cytokines [10–13]. As a modulator of NO-dependent macrophage cytotoxicity, arginase I is implicated in the regulation of macrophage activity in wound healing [14] and the suppression of the tumoricidal activity of macrophages [15] and T cells [16]. Recently, our group demonstrated that arginase I inhibition reduces endothelial dysfunction and blood pressure rising in spontaneously hypertensive rats [17]. An interesting feature observed in the active site of this enzyme is the presence of a catalytically important, non-coordinating histidine residue. The crystal structure of rat arginase I reveals that the enzyme contains a $Mn_2{}^{2+}$ cluster bridged by a water molecule/hydroxide ion believed to be the catalytic nucleophile [18–20]. pH rate profiles for rat arginase I indicate that a Hist 141 must be deprotonated for maximal catalytic activity [21]. Arginase I contains a histidine residue, Hist 141, located partway out of the active site cleft and 4.2 Å from the metal-bridging solvent molecule. Residue Hist 141 is strictly conserved in all arginases, as well as in the arginase family-members agmatinase and proclavaminate amidino hydrolase [22]. A variety of data implicate Hist 141 in catalysis: (i) arginase I is inactivated by treatment with diethyl pyrocarbonate (DEPC), but Hist 141 → Asn arginase I (i.e., Hist 141 N arginase I) is unaffected [23], (ii) *N*-bromosuccinimide (NBS) inactivates arginase I at Hist 141 [24], (iii) the Hist 141 N arginase I variant displays 11% of wild-type activity [23], (iv) Hist 141 → leucine (L) human arginase I (i.e., Hist 141 L human arginase I) exhibits 2.6% activity as compared to a wild-type control [25], (v) human arginase I is inactivated by DEPC and photoinactivated by rose Bengal, while Hist 141 → phenylalanine (F) arginase (i.e., Hist 141 F arginase) is unaffected by these treatments [26], and (vi) human arginase I is inactivated by Woodward's reagent K at Hist 141 [27]. Interestingly, Hist 141 F human arginase I and Hist 141 N rat arginase I exhibit only modest ($\leq$10-fold) changes in K_M values, so a significant interaction of Hist 141 with substrate arginine is unlikely [7,23,26]. The crystal structure of rat arginase I complexed with a boronic acid inhibitor shows that Hist 141 hydrogen bonds to a water molecule which in turn donates a hydrogen bond to the α-carboxylate group of the inhibitor [28]. Consistent with these data, it is proposed that Hist 141 serves as a proton shuttle that helps regenerate the nucleophilic metal-bound hydroxide ion for catalysis [18], analogous to Hist 64 of carbonic anhydrase [29,30]. The technique usually employed to immobilize enzymes on solid supports are mainly based on chemical mechanisms. These chemical immobilization methods mainly include enzyme attachment by covalent bonds between enzyme and matrix. The most widely used method is based on the activation of amino supports, independently of their nature: porous [31–38], siliceous [39–43], polymeric [44], or monolithic [45,42]. The arginase enzyme has been immobilized by employing *N*,*N*′-disuccinimidylsuberate (DSS) as activating agent [39,40]. This novel chromatographic support was used to determine and quantify the forces driving association between N^{ϖ}-hydroxy-nor-L-arginine (nor-NOHA) which is a very good arginase inhibitor [46] and the bovine liver arginase I enzyme. The energetic of binding of the inhibitor to the enzyme as both a function of temperature and pH was studied using this novel biochromatographic approach. Those experiments allowed us also to calculate the number of protons linked to ligand binding and to probe the catalytic function of Hist 141.

2. Experimental and method

2.1. Reagents

Water was obtained from an Elgastat option water purification system (Odil, Talant, France) fitted with a reverse osmosis cartridge. nor-NOHA was obtained from Bachem (Germany) and crystalline bovine liver arginase I was obtained from Sigma–Aldrich (Paris, France). *N*,*N*′-disuccinimidyl suberate