

シトソール型アデニレートキナーゼとニコチンアミドアデニンジ ヌクレオチドとの相互作用

誌名	Japanese journal of veterinary science
ISSN	00215295
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巻/号	50巻2号
掲載ページ	p. 509-512
発行年月	1988年4月

Interaction between Cytosolic Adenylate Kinase and Nicotinamide Adenine Dinucleotide

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(Received 26 October 1987/Accepted 23 December 1987)

ABSTRACT. Cytosolic adenylate kinase (AK1) was eluted with a high yield from an affinity column of blue dextran-Sepharose 4B which bound AK1 with NADH at a low concentration but not with NAD⁺ at the same concentration. The difference spectrum of AK1-NADH complex against free AK1 and NADH showed positive maxima at 269nm and 273nm and a negative maximum around 326nm, and that of AK1-NAD⁺ complex also showed a positive maximum at 275nm. NADH and NAD⁺ competitively inhibited AK1 with respect to both AMP and ATP. From these results and reference data describing on topological equivalence between substrate-binding sites of AK1 and NAD-binding site of dehydrogenases, we can speculate that NAD adenine and nicotinamide moieties bind to ATP and AMP-binding sites of an AK1 molecule, respectively.—**KEY WORDS:** adenylate kinase, NAD, nucleotide-binding site.

Jpn. J. Vet. Sci. 50(2): 509–512, 1988

Adenylate kinase (ATP:AMP phosphotransferase, EC 2.7.4.3, AK) is a small monomeric enzyme which catalyzes the reaction $ATP + AMP \rightleftharpoons 2ADP$ [1, 6]. The three-dimensional structure of porcine cytosolic AK (muscle type AK, AK1) is known [5]. AK1 binds to an affinity column of blue dextran-Sepharose, which has been used to purify the enzymes such as kinases and dehydrogenases [4, 9]. During examining the conditions which elute AK1 from the affinity column, we found that NADH as well as substrates for AK is a useful eluant. This reminded us of a topological similarity of the substrate-binding sites in AK1 to the NAD-binding site in dehydrogenases [3, 7]. Here, we describe the interaction of NAD with AK1.

MATERIALS AND METHODS

Routine enzyme assay was carried out as described previously [10]. AK1 was purified from rat skeletal muscle essentially by the

method of Noda *et al.* [2] for carp muscle AK, and the purified enzyme had a specific activity of 3,970 U/mg protein. The purified rat AK1 was determined using an absorption coefficient of $A_{279nm}^{1\%} = 5.6 \text{ cm}^{-1}$. Blue dextran-Sepharose 4B was prepared according to the procedure of Ryan and Vestling [4].

The initial velocities of AK1 reaction were determined by the chromatographic assays. The reaction mixtures (1ml, pH7.5) contained 100 mM triethanolamine-HCl buffer, 2 mM MgSO₄, ATP, AMP, inhibitor (as indicated in Fig. 3), 0.1 mg/ml bovine serum albumin, 1 mM 2-mercaptoethanol, and 1.3 ng/ml AK1. The reactions were initiated by the addition of AK1 and allowed to proceed for appropriate periods of time at 30°C. 500μl of 1.2 M perchloric acid was added to the reaction mixture to terminate the reaction, and the samples were allowed to stand in ice for 1 hr. Proteins were precipitated by centrifugation at 1,600xg for 20 min at 2°C, and aliquots

(800 μ l) of the supernatants were removed and neutralized by the addition of 200 μ l of 1.6 M KOH. After precipitating insoluble potassium perchlorate by centrifugation, nucleotides in the supernatants were separated by high-performance liquid chromatography on a TSK gel DEAE-2SW column (4.6 \times 250mm, Toyo Soda Manufacturing Co., Ltd.) by elution with 0.1 M sodium phosphate buffer, 20% acetonitrile, pH7.0, at a flow rate of 0.7 ml/min and detected by absorbance at 259nm.

RESULTS AND DISCUSSION

Fig. 1 shows the elution pattern of AK1 from AK1-binding blue dextran-Sepharose 4B column by a linear 0–10 mM gradient of NAD. More than 90% of the AK1 activity bound to the column was eluted by NADH, whereas less than 2% of the bound activity

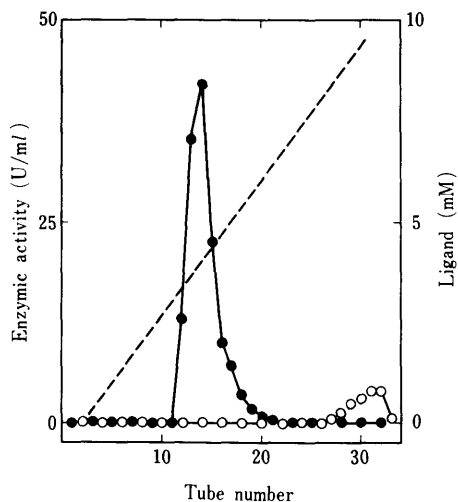


Fig. 1. Elution patterns of AK1 from the blue dextran-Sepharose 4B column by NAD. The AK1 solution of 300 U was applied to a column (9 \times 55mm) equilibrated with 10 mM Tris-HCl (pH7.5) and the activity was eluted by a linear gradient obtained by mixing 30ml of the same buffer and 30ml of 10 mM NADH (●) or NAD⁺(○) at a flow rate of 30 ml/hr. Fractions of 2ml were collected and the enzymic activity was measured. ---, ligand gradient.

was eluted by NAD⁺. Using substrates of AK1 as eluants, ATP and AMP eluted more than 90% of the bound activity at concentrations of 1 mM and 10 mM, respectively. Sodium chloride eluted the AK1 from the column at a concentration of higher than 200 mM. These results indicate that the elution of AK1 by NADH is ligand-specific as well as the substrates, but not owing to its ionic strength [9].

The interaction of NAD with AK1 was also demonstrated by spectrophotometric method. Measurement of the ultraviolet-difference spectrum of the AK1-NADH complex against AK1 and NADH showed two positive peaks at 269nm and 273nm and a negative peak around 326nm (Fig. 2-a). The difference spectrum of AK1-NAD⁺ complex also showed a positive peak at 275nm (Fig. 2-b).

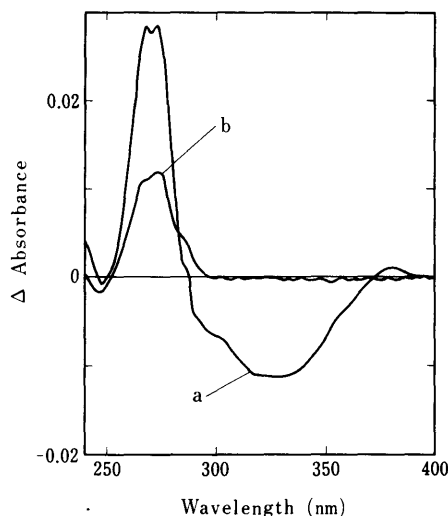


Fig. 2. Ultraviolet-difference spectra of AK1-NAD complex against AK1 and NAD. Measurements were made at room temperature using tandem cuvettes: the path length of each compartment of a cuvette was 4.5mm. The sample cuvette contained the AK1-NAD complex in 10 mM Tris-HCl, 0.5 mM 2-mercaptoethanol, pH7.5, in one compartment and buffer alone in the other. The reference cuvette contained AK1 in one compartment and NAD in the other, both in the same buffer. The concentrations of AK1, NADH (a) and NAD⁺ (b) were 31.0 μ M, 66.7 μ M and 71.4 μ M, respectively.

The kinetic studies of inhibitions of AK1 by NADH and NAD⁺ showed that competitive inhibitions to both AMP and ATP substrates occurred by both compounds, and that NADH was a stronger inhibitor than NAD⁺ (Fig. 3). Neither oxidized form nor reduced form of nicotinamide mononucleotide significantly inhibited AK1 activity at a concentration of 1 mM (data not shown).

From the above results, it is considered that the adenine and nicotinamide moieties of NAD bind to the substrate ATP and AMP-binding sites of AK1. However, we could not elucidate the binding manner of NAD to AK1 by the only data obtained here. Three-dimensional topological comparison of AK1 with several dehydrogenases suggested that ATP adenine-binding site and AMP adenine-binding site of AK1 may correspond to NAD adenine-binding site and NAD nicotinamide-binding site of dehydrogenases, respectively [3, 7]. Therefore, we can speculate that the ade-

nine and nicotinamide parts of NAD bind to the ATP and AMP-binding sites of AK1, respectively.

Storey [8] reported an inhibitory effect of a physiological concentration of NADH on squid mantle AK and proposed the metabolic control of the enzyme by NADH: inhibition of AK occurs at a high NADH concentration during resting muscle and deinhibition of AK occurs by rapid consumption of NADH through muscle contraction. The results of the present study indicate that NADH binds to AK1 with a higher affinity than NAD⁺. Therefore, there may be the possibility that mammalian AK1 is metabolically modulated by the fluctuation of NADH/NAD⁺ ratio coupled with muscle exercise.

ACKNOWLEDGEMENTS. This research was supported by a Grant-in-Aid for Scientific Research (No. 61760268) from the Ministry of Education, Science and Culture of Japan, and by a Research Grant (No. 5906) from School of Veterinary Medicine and Animal Sciences, Kitasato University.

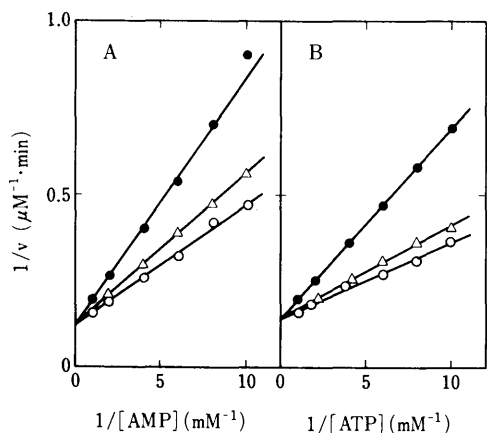


Fig. 3. Double-reciprocal plots of the catalytic activity of AK1 in the presence and absence of NAD. The initial velocities were determined by the chromatographic assays described in the text and expressed as the concentrations of ADP formed per min ($\mu\text{M}/\text{min}$). (A) and (B) show the dependence of $1/v$ upon the reciprocal of the concentration of AMP (with 1 mM ATP) and ATP (with 1 mM AMP), respectively. (○), no inhibitor; (●), 1 mM NADH; (Δ), 1 mM NAD⁺.

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要 約

シトソール型アデニレートキナーゼとニコチンアミドアデニンジヌクレオチドとの相互作用：渡辺清隆・田中一彦・小島 浩・今西隆和・山本晋二（北里大学獣医畜産学部獣医生理化学教室）——ブルーデキストラン-Sepharose 4B アフィニティカラムに結合したシトソール型アデニレートキナーゼ（AK1）は、低濃度の NADH により高収率で溶出されたが、 NAD^+ によっては溶出されなかった。AK1-NADH 複合体の差スペクトルは、269nm および 273nm に正の極大を、326nm 付近に負の極大を示し、また、AK1-NAD⁺ 複合体のそれも、275nm に正の極大を示した。NADH および NAD^+ は、AMP および ATP の両基質に関して、AK1 を競争阻害した。これらの結果から、NAD のアデニンおよびニコチンアミド部分は、AK1 の ATP 結合部位および AMP 結合部位にそれぞれ結合すると想像された。