Bovine Cytosolic 5’-Nucleotidase Acts through the Formation of an Aspartate 52-Phosphoenzyme Intermediate*

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Cytosolic 5’-nucleotidase/phosphotransferase (cN-II), specific for purine mononucleotides and their deoxyderivatives, acts through the formation of a phosphoenzyme intermediate. Phosphate may either be released leading to 5’-mononucleotide hydrolysis or be transferred to an appropriate nucleoside acceptor, giving rise to a mononucleotide interconversion. Chemical reagents specifically modifying aspartate and glutamate residues inhibit the enzyme, and this inhibition is partially prevented by cN-II substrates and physiological inhibitors. Peptide mapping experiments with the phosphoenzyme previously treated with tritiated borohydride allowed isolation of a radiolabeled peptide. Sequence analysis demonstrated that radioactivity was associated with a hydroxymethyl derivative that resulted from reduction of the Asp-52-phosphate intermediate. Site-directed mutagenesis experiments confirmed the essential role of Asp-52 in the catalytic machinery of the enzyme and suggested also that Asp-54 assists in the formation of the acyl phosphate species. From sequence alignments we conclude that cytosolic 5’-nucleotidase, along with other nucleotidas, belong to a large superfAMILY of hydrolases with different substrate specificities and functional roles.

Hydrolysis of the phosphate esterified in 3’ or 5’ of mononucleotides is catalyzed by a family of nucleotidases whose members differ in terms of substrate specificity, cellular location, regulation, distribution, and amino acid sequence. A better knowledge of the structure and catalytic mechanism of all these proteins is necessary for the understanding of their origin, evolution, and physiological significance. A classification of the enzymes specific for purine 5’-mononucleotides has been proposed by Zimmerman on the basis of cellular location and physiological significance. A family of hydrolases with different substrate specificities and functional roles.

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1 The abbreviations used are: cN, cytosolic nucleotidase; TPCK, L-1-II. Moreover, other nucleotidas specific for 5’- and 3’-deoxyribonucleotides located in cytosol (dNT-1) or in mitochondria (dNT-2) have been described (2, 3). Furthermore, 5’-nucleotidas specific for pyrimidine mononucleotides have been described in human erythrocytes (PN-I and PN-II) (4, 5). Recently, PN-I has been found to be identical to p36, an α-interferon-induced protein playing a role in immune diseases (6). All these proteins expressing nucleotidase activity do not show significant sequence homologies to each other (7, 8).

 cN-II has been demonstrated to act also as a phosphotransferase, which is a consequence of its reaction mechanism, proceeding through the formation of a phosphoenzyme covalent intermediate (9, 10). No phosphotransferase activity has been reported for the other nucleotidas described so far with the exception of PN-I and PN-II (11). cN-II is a widely expressed enzyme with a remarkable sequence conservation through evolution. Its activity is involved in the regulation of the availability of intracellular IMP (12). Because IMP is the precursor of all purine nucleotidas, which are not only nucleic acid building blocks but also energy transducers, intracellular and extra cellular signals, and metabolic regulators, the regulation of its hydrolysis is of paramount importance for a number of cell functions. Recently, an increased activity of cN-II has been associated to a developmental neurological disorder and to Lesch-Nyhan syndrome (13, 14).

In this work we provide evidence that cN-II becomes phosphorylated during the catalytic cycle on the first aspartate (Asp-52) occurring in a DMDYT motif conserved among the different species. This consensus sequence is also shared with other members of the 5’-nucleotidas family. In addition, a DXDX(T/V) motif has been described as being involved in the catalytic mechanism of a number of other phosphotidas/phosphotransferas (15), suggesting a common mechanism of action for all these enzymes that do not present any significant sequence homology with cN-II.

EXPERIMENTAL PROCEDURES

Chemicals—[14C]Clinosine (56.1 mCi/mmol), N-ethyl-5-phenylisoxazolium-3-sulfonate (Woodward’s reagent K) and TPCK-treated trypsin were purchased from Sigma; [3H]NaBH4 (11.4 Ci/mmol) and [32P]AMP (3 Ci/mmol) were purchased from Amersham Pharmacia Biotech. Taq DNA polymerase was purchased from MBI Fermentas, OptiPhase ‘HiSafe’ 3 scintillation mixture was purchased from Wallac. [32P]IMP (33526 This paper is available on line at http://www.jbc.org

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zyme 5 min before the addition of WRK: 20 mM MgCl₂, 2 mM IMP, 5 mM ATP, and 10 mM sodium phosphate. After 4 min of incubation with 400 nM WRK the enzyme was assayed for residual activity. The experiments were performed in triplicate, and the standard deviation was less than ±5% of the mean value.

Enzyme Preparation and Assay—Bovine recombinant cytosolic 5′-nucleotidase was prepared as previously described (16), and its concentration was determined by the method of Bradford (17) using bovine serum albumin as standard. Molar concentration of the enzyme was calculated using the molecular mass of its subunit (66 kDa). Phosphotransferase activity was assayed according to Tozzi et al. (18). Briefly, the reaction mixture in 50 mM Tris-HCl, pH 7.4, contained 1.4 mM ATP, 0.2 mM IMP, 4.5 mM ATP or BPG, 1 mM dithiothreitol. If not differently specified, cN-II was assayed at a final concentration of 3–6 nM (corresponding to 0.02–0.15 μM) in 50 μl of reaction mixture.

Treatment of cN-II with Woodward’s Reagent K.—Freshly prepared stock solutions of WRK (0.2–22 mM) in ice cold 1 mM HCl were used. The inactivation reactions were performed at 25 °C in 0.2 mM MES buffer, pH 6.0. The reaction mixture contained: 1 μM purified cN-II and WRK ranging from 0 to 400 μM. At 0, 1, 2, 3 and 4 min, 10 μl aliquots were withdrawn from the reaction mixture, diluted in 5 mM Tris-HCl, pH 7.4, and then assayed for phosphotransferase activity. Protection against inactivation was assessed with the following ligands before addition of WRK: 20 mM MgCl₂, 2 mM IMP, 5 mM ATP, and 10 mM sodium phosphate. After 4 min of incubation with 400 nM WRK the enzyme was assayed for residual activity. The experiments were performed in triplicate, and the standard deviation was less than ±5% of the mean value.

Chemical Entrainment of the Acyl Phosphate Intermediate—Bovine

FIG. 1. Inactivation of cN-II by Woodward’s reagent K. A, cN-II (1 μM) was treated with 0 ( ), 5 ( ), 20 ( ), 100 ( ), 175 ( ), 300 ( ), and 400 ( ) μM WRK as described under “Experimental Procedures.” At various time intervals, aliquots were withdrawn and assayed for residual activity. B, concentration dependence of the first-order rate constant, Kobs.

C/mmol) was obtained from [32P]AMP as previously described (10).

5′-nucleotidase (190 μg, 2.8 nmol) was incubated in 150 μl of reaction buffer containing no (control) or 5.3 mM IMP, 27 mM MgCl₂, 6 mM ATP, 2.6 mM dithiothreitol, and 50 mM Tris-HCl, pH 7.4, at 0 °C. The reaction was stopped after 30 s by the addition of 10% ice-cold trichloroacetic acid (750 μl). After centrifugation (10 min at 12000 × g and 4 °C), the pellet was resuspended in 750 μl of 5% ice-cold trichloroacetic acid and centrifuged for 10 min at 4 °C. The pellets were washed with 10 μl ice-cold HCl, centrifuged for 5 min at 4 °C, and lyophilized. Protein samples obtained from the labeling experiment and control were independently resuspended in 65 μl of MeSO containing 25 mCi of [3H]NaBH₄ (11.4 Ci/mmol) and incubated at room temperature. After 10 min, 10 μmol of unlabeled NaBH₄ (solved in 35 μl of MeSO) were successively added and incubated at room temperature for other 10 min. One milliliter of 0.44 mM ice-cold HClO₄ was added to both reaction mixtures, and samples were placed on ice for 30 min and then centrifuged for 20 min at 12000 × g and 4 °C. The resulting pellets were washed with 500 μl of cold acetone and lyophilized.

Preparation and Isolation of Tryptic Peptides—Protein samples were desalted and concentrated by a 12% mini SDS-polyacrylamide gel electrophoresis (20); gels were stained with Coomassie Blue R250 and destained for 3 h with 10% acetic acid. Protein bands were excised, and gel pieces were tritured, washed with water, reduced with dithiothreitol and carbamidomethylated (21). Gel pieces were extracted by sonication with 500 μl of 0.4% NH₄HCO₃/acetoni trile 1:1 v/v, pH 8.5 (twice). Extracts were combined and lyophilized.

Peptide mixtures were fractionated by reverse-phase HPLC on a Vydac C₄ column 218TP52 (250 × 2.1 mm), 5 μm, 300 Å pore size (The Separation Group) by using a linear gradient from 5–60% of acetonitrile in 0.1% trifluoroacetic acid over 60 min at flow rate of 0.2 mL/min. Individual components were manually collected and dried in a Speed-
PCR 2: no template, 100 pmol of P2R with the same cycling conditions. Primers were as follows: FnheI, 5'-CCGCTAGCATGACAACCTCC-3' (from base 8 to base 14 of pET28c-5'term construct); P2R, 5'-CCCAAAATGTTAGCTCCAATAA-3', from base 1065 to 1044); D52-R1, 5'-TGTTATTACCATGNYCCTAAACCCA-3' (from base 168 to 147); D54-R1, 5'-TGTTATTAYCCATGCTAAAAACCCA-3' (from base 168 to 147).

Preparation of 32P-Enzyme Covalent Intermediate—About 1.5 µg of cN-II (wild type or mutants) were incubated 150 s on ice in 15 µl of a solution of 20 mM MgCl2, 7.2 mM ATP, 1.5 mM [32P]IMP, and 2 mM dithiothreitol. The reaction was stopped by adding 5 µl of 8% SDS in 0.25 M Tris-HCl, pH 6.8; the samples were vortexed and loaded on a nitrocellulose membrane (0.2 µm-Sartorius). The membrane was washed for 8 min with 100 ml of 2% SDS in 60 mM Tris-HCl, pH 7.4 (5 times), and radioactivity was measured with 5 ml of scintillation mixture.

RESULTS

5'-Nucleotidase Inactivation by Woodward’s Reagent K—To get information on the nature of the amino acid residues occurring in the active site of 5'-nucleotidase, different inhibitors were tested as effectors of the enzyme activity. Among these, carboxylate-directed compounds resulted to inhibit the enzyme. In fact, incubation of recombinant protein with Woodward’s reagent K, at pH 6.0 resulted in a time- and concentration-dependent loss of activity (Fig. 1A). A plot of the observed rate constants (Kobs) versus reagent concentration over the range 5–400 µM yielded a second order rate inactivation constant of 1.2 mM−1 m−1 (Fig. 1B). The effect on the chemical modification by substrates or inhibitors of the enzyme, was studied. Fig. 2 shows that among the different compounds tested, the allosteric inhibitor phosphate either in the absence or in the presence of Mg2+ exerted the highest protection against enzyme inactivation. Similarly, IMP (the best cN-II substrate) also provided a good protection, which was increased by the presence of Mg2+. In contrast, the allosteric activator ATP in combination with Mg2+ was unable to protect the enzyme in the absence of the substrate. However, addition of IMP and phosphate to this mixture resulted again in a substantial protection from chemical inactivation. Fig. 3 shows the stoichiometry of WRK incorporation into cN-II as a function of residual activity. The plot is monophasic, and the experimental data fit a straight line that intersects the abscissa at a point corresponding to the incorporation of 4.1 mol of WRK/mol of enzyme.

Identification of Phosphorylated Residue in cN-II—The experiments reported above indicated that carboxyl groups are involved in the catalytic mechanism of the enzyme. Because the hydrolysis of the monophosphate catalyzed by cN-II proceeds through the formation of a phosphoenzyme intermediate (10), we attempted to trap this derivative to identify the residue involved in catalysis. However, phosphoseryl and phosphothreonyl residues are too labile to resist to the procedures commonly used to identify phosphorylated residues (enzymatic digestion of the protein, separation of the resulting peptides, and sequencing of the phosphorylated species). Therefore, we used the method of Degani and Boyer (23) in which the unstable carboxyl phosphate adduct is reduced with [3H]NaBH4 to a stable radiolabeled hydroxymethyl-containing derivative before further processing of the protein. Peptide mapping, radioactivity measurement, and mass spectrometric analysis of the fractions obtained allowed a direct identification of the phosphorylated residue.

The recombinant enzyme incubated with or without (control) inosine-monophosphate was treated with radioactive borohydride, denatured, and concentrated by a mini-SDS-polyacrylamide gel electrophoresis (20). Gel bands were reduced, alkylated, and digested in situ with trypsin as previously reported (21). The resulting digests were separated by reverse phase-HPLC as illustrated in Fig. 4 for the labeled protein (panel B) and control (panel C). A radioactive component was simulta-
neously present in both cases (PL#3 and PC#3). This fraction eluted at the same retention time (10.4 min) as [3H]NaBH₄ (determined in a control chromatogram) and did not yield any significant signal when analyzed by MALDIMS. On the contrary, a second radioactive fraction (PL#29) eluting at 23.8 min was observed in the case of the labeled protein. When analyzed by MALDIMS, it showed the presence of three peptides as reported in Fig. 5. The species with MH⁺ at 1364.6 m/z was assigned as the peptide fragment 292–303. The remaining components presented clear signals at 1716.9 and 1731.1 m/z, respectively. The latter was associated to the carboxamidomethylated peptide 48–61; the former did not correspond to any predicted species with regard to its molecular mass. On the basis of the expected partial conversion of its parent carboxylate to hydroxymethyl group, the presence of a satellite species differing for −14 Da was indicative of the polypeptide where phosphorylation occurred during catalysis. Therefore, peptide 48–61 apparently contained the active site residue of 5′-nucleotidase.

To ascertain the identity of this residue (discriminating be-
tween Asp-52 and Asp-54 present in the peptide sequence), fraction PL#29 was submitted to sequence analysis. A modified sequencer program allowed to collect a portion of PTH-derivative released at each cycle of Edman degradation successively measured for radioactivity. The results obtained are shown in Table I. Two different sequences were simultaneously detected, which confirmed that the peptides present in this radioactive fraction were those predicted by the MALDIMS experiments. The radioactivity recovered at each cycle of degradation indicated that the majority was observed in the position corresponding to Asp-52 (cycle 5). A significant amount of PTH-derivative-Hse was found at this cycle, demonstrating the conversion of the carboxyl phosphate group of Asp-52 to the [3H]hydroxymethyl group of Hse. A negligible amount of radioactivity was observed in the fraction from the cycle corresponding to Asp-54 (cycle 7). The absence of a satellite signal at 14 Da from peptide 292–303 and the low amount of radioactivity recovered at the cycle, where the only acid residue (Glu-298) present in the sequence occurred, ruled out the possibility that this peptide was the labeled one. Therefore, these experiments definitively demonstrated that the residue phosphorylated during the reactions catalyzed by 5'-nucleotidase is Asp-52.

**Site-directed Mutagenesis of Cytosolic 5'-Nucleotidase**—To substantiate the active-site labeling experiments, we constructed cN-II mutants in which we replaced separately Asp-52 and Asp-54 by either glutamate or alanine residues. In addition, we prepared a mutant where 42 amino acid residues at the N terminus were deleted, taking advantage of a restriction site specific for NcoI present in the nucleotide sequence. Extracts prepared from transformed cultures were tested for nucleotidase activity, as reported in Table II. Only the wild type enzyme was active; all mutants displayed an activity comparable with that observed in a control culture without a translatable insert. All of these extracts were chromatographed on a Talon ion metal-affinity chromatography column, yielding comparable amount of purified proteins as determined by SDS-polyacrylamide gel electrophoresis analysis (result not shown). Among the purified proteins, again only the wild type enzyme was active, and its specific activity indicated that by Talon chromatography a 10-fold purification was obtained (Table II). When the purified proteins were incubated in the presence of [32P]IMP, only the wild type enzyme became radioactive, indicating that a certain amount of phosphorylated intermediate was formed during catalysis; on the contrary, the mutant proteins were completely devoid of radioactivity (Table II).

**Sequence Comparison**—The result mentioned above demonstrated that Asp-52 is the amino acid residue of cN-II that is phosphorylated during catalysis; in addition, Asp-54 seems to assist in the formation of this covalent intermediate. Both
residues are present in a DMDYT motif highly conserved among cN-IIs from different species. Although a BLAST search in different data banks revealed that cN-II do not present a significant sequence identity or homology with other proteins, a careful analysis revealed that other polypeptide species are characterized from this specific signature. Fig. 6 shows the alignment of the cN-II DMDYT motif with that occurring in other members of the 5'-nucleotidase family, such as PN-I (specific for pyrimidine mononucleotides), both cytosolic and mitochondrial 5'-3'-nucleotidases dNT-1 and dNT-2 (specific for deoxynucleotides), and cN-I (AMP-specific cytosolic 5'-nucleotidase). Membrane-bound 5'-nucleotidase do not contain this conserved region.

Several other phosphatases and phosphotransferases have been reported to form a covalent phosphointermediate involving an aspartate residue (24, 25). Recently, a new class of phosphatases or phosphotransferases have been described as containing a conserved DXDX(T/V) motif (15, 26) (Fig. 6). Also for several members of this large family it was demonstrated that the first aspartate occurring in this region becomes phosphorylated during catalysis (15). Although these enzymes show minimal sequence homology to cN-II (less than 5%), they all contain the consensus sequence described in this manuscript.

**DISCUSSION**

The results reported in this work demonstrate that cN-II is inhibited by WRK in a dose-dependent manner, suggesting that this reagent modifies carboxylate residues important for proper enzymatic functioning. WRK is well established in terms of its ability to modify aspartates or glutamates (27, 28). The selection of appropriate reaction conditions (pH 6.0 and very short inactivation times) ensured us an increased selectivity of WRK for acid residues and ruled out a possible reaction of this compound with other nucleophile amino acids (29–31).

The partial protection exerted by substrates and inhibitors of the enzyme is strongly indicative that some of the modified residues are located in the active site. It was previously observed that the allosteric inhibitor phosphate causes an increase in the $$K_m$$ for IMP, suggesting that the cN-II active site can result less accessibly in the presence of this anion. On the contrary, has been reported that the allosteric activator ATP, in the presence of Mg$$^{2+}$$ causes a moderate decrease in the $$K_m$$ for the substrate and an increase in the catalytic rate (32). We find here that phosphate is effective in protection against WRK inactivation, while ATP-Mg$$^{2+}$$, which increase the accessibility of the active site, do not exert any protective effect. On the contrary, ATP in the absence of Mg$$^{2+}$$ indeed afforded a protection similar to that observed for IMP. ATP is also stabilizing the enzyme during storage and against thermal inactivation (12). These observations indicate that, in the absence of Mg$$^{2+}$$, ATP can determine a protective effect possibly by an interaction with the active site.

In a previous paper we demonstrated that cN-II forms an Asp-52-Phosphointermediate by guest on July 24, 2018http://www.jbc.org/Downloaded from
acceptor; similarly, Asp-13, Asp-167, and Glu-20 seem to
four-helix-bundle domains. A careful structural analysis re-
lates described by Collet et
strate that cN-II belongs to the large class of phosphohydro-
generate this intermediate. On this basis, our results demon-
and prevented the formation of the phosphorylated enzyme. In
substitution in this position totally abolished enzyme activity
residue for the enzyme activity; in fact, even a conservative
analysis performed on the isolated peptide demonstrated that
radiolabeled borohydride, and separation of its tryptic digest,
tained after reduction of the acyl phosphate intermediate with

Asp-11 and Asp-13), are essential for ca-
motif of cN-II (Asp-52 and Asp-54), similar to that of phospho-
site. In addition, both Asp residues occurring in the DXDX(T/V)
suggesting that modified residues are located into the active

formalism. Therefore, these data are in strict analogy with that
serine phosphatase (Asp-11 and Asp-13), are essential for ca-

Furthermore, sequence comparison of cN-II with other nucle-
oses demonstrate that cN-II is a first example of a group of eukary-
olic cytosolic nucleotidases presenting a common catalytic ma-

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REFERENCES

1. Zimmerman, H. (1992) Biochem. J. 285, 345–365
2. Hoglund, L., and Reichard, P. (1996) J. Biol. Chem. 271, 6589–6595
3. Rampazzo, C., Gallinaro, L., Milanese, E., Freginellina, E., Reichard, P., and
4. Valentine, W. N., Pink, K., Paglia, D. E., Harris, S. R., and Adams, W. S. (1974) J.
5. Hirono, A., Fujii, H., Natori, H., Kurokawa, I., and Miwa, S. (1987) Br. J. Hae-
6. Amici, A., Emanuelli, M., Raffaelli, N., Ruggieri, S., Sacrifice, F., and Maggi, G.
7. Sala-Newby, G. B., Skladanowski, A., and Newby, A. C. (1999) J. Biol.
8. Rampazzo, C., Johansson, M., Gallinaro, L., Ferraro, P., Hellman, U., Karlsson, A.,
9. Warku, Y., and Newby, A. C. (1982) Biochem. J. 205, 503–510
10. Baiocchi, C., Pesi, R., Camici, M., Itoh, R., and Tozzi, M. G. (1996) Biochem. J.
11. Amici, A., Emanuelli, M., Maggi, G., Raffaelli, N., and Ruggeri, S. (1997) FEBS Lett.
12. Itoh, R., Echizen, H., Hisuchi, M., Oka, J., and Yamada, K. (1992) Comp.
13. Page, T., Yu, A., Fontanesi, J., and Nyhan, W. L. (1997) Proc. Natl. Acad. Sci.
14. Pesi, R., Micheli, V., Jacocelli, G., Peruzzi, L., Camici, M., Garcia-Gil, M.,
15. Collet, J. F., Strobhant, V., Pirard, M., Delpierre, G., and Van Schaftingen, E.
16. Allegrini, S., and Tozzi, M. G. (1994) J. Biol. Chem. 269, 24508–24516
17. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
18. Tozzi, M. G., Camici, M., Pesi, R., Allegrini, S., Sgrarrella, F., and Ipatria, P.
19. Shina, U., and Brever, J. M. (1985) Anal. Biochem. 151, 327–333
20. Vanderkolk, J., Rider, M., Rasmussen H. H., De Boek, S., Puyre, M., Van
21. Keresztessy, Z., Kiss, L., and Hughes, M. A. (1994) Arch. Biochem. Biophys.
22. Technical Tips
23. Marisich, E., Bandiera, A., Tell, G., Sealoni, A., and Mannini, G. (2001) Eur.
24. Ekici, A. B., Park, O. S., Fuchs, C., and Rautensrauss, B. (1997) Technical Tips
25. Shina, U., and Brever, J. M. (1985) Anal. Biochem. 151, 327–333
26. Vanderkolk, J., Rider, M., Rasmussen H. H., De Boek, S., Puyre, M., Van
27. Keresztessy, Z., Kiss, L., and Hughes, M. A. (1994) Arch. Biochem. Biophys.
28. Kommisarov, A. A., Romanova, D. V., and Debabov, V. G. (1995) J. Biol.
29. Maralihalli, G. B., and Bhagwat, A. S. (1993) Biochem. J. 285, 1055–1065
30. Rampazzo, C., Johansson, M., Gallinaro, L., Ferraro, P., Hellman, U., Karlsson, A.,
31. Rampazzo, C., Johansson, M., Gallinaro, L., Ferraro, P., Hellman, U., Karlsson, A.,
32. Rampazzo, C., Johansson, M., Gallinaro, L., Ferraro, P., Hellman, U., Karlsson, A.,
33. Rampazzo, C., Johansson, M., Gallinaro, L., Ferraro, P., Hellman, U., Karlsson, A.,
34. Rampazzo, C., Johansson, M., Gallinaro, L., Ferraro, P., Hellman, U., Karlsson, A.,
35. Rampazzo, C., Johansson, M., Gallinaro, L., Ferraro, P., Hellman, U., Karlsson, A.,
36. Rampazzo, C., Johansson, M., Gallinaro, L., Ferraro, P., Hellman, U., Karlsson, A.,
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