Volume 244, number 1, 123-126 FEB 06791 February 1989

A specific, low K_m ADP-ribose pyrophosphatase from rat liver

Asunción Miró*, María Jesús Costas, Miguel García-Díaz, María Teresa Hernández and José Carlos Cameselle

Departamento de Bioquímica y Biología Molecular y Genética and **Departamento de Patología y Clínicas Humanas (Médicas), Facultad de Medicina, Universidad de Extremadura, Instituto de Investigaciones Biomédicas del Consejo Superior de Investigaciones Científicas, E-06080 Badajoz, Spain*

Received 3 December 1988

Two rat liver ADP-ribose pyrophosphatases (ADPRibases) were partially purified. ADPRibase-I hydrolyzed ADP-ribose ($K_m = 0.5$ μM) giving AMP as a product, required Mg^{2+} or, less efficiently, Mn^{2+} (Ca^{2+} was not active), its activity changed little between pH 7 and 9, and was specific for ADP-ribose as it did not hydrolyze ADP-glucose, NAD^+, NADH or diadenosine 5',5'''-P^1,P^n-*n*-phosphates (Ap_2A, Ap_3A). ADPRibase-II showed similar properties, except that the K_m for ADP-ribose was 50 μM and may be non-specific, as the same preparation hydrolyzed ADP-glucose, NADH and Ap_2A. ADPRibase-I fulfils the requirements of a specific turnover pathway consistent with a cellular role for free ADP-ribose.

ADP-ribose pyrophosphatase; ADP-ribose, free; ADP-ribose turnover; ADP-ribosylation; (Liver)

1. INTRODUCTION

Free ADP-ribose can be formed in vitro by NAD- or poly(ADP-ribose) glycohydrolases related to the turnover of NAD or protein-linked poly(ADP-ribose) [1-6]. Whether ADPRib has a specific cellular role is not known but, for instance, nonenzymic glycosylations by ADPRib occur in vitro and could happen in vivo [7-11]. As ADPRib catabolic enzymes have not been studied in detail, it is not known whether ADPRib turnover occurs through a specific pathway, which could be consistent with a cellular role for the free metabolite. Driven by previous work with other specific and unspecific phosphohydrolases [12], we have investigated this point and found two rat liver pyrophosphatases, one of which is specific for ADPRib with a K_m value of 0.5 μM.

Correspondence address: J.C. Cameselle, Departamento de Bioquímica y Biología Molecular y Genética, Facultad de Medicina, Universidad de Extremadura, E-06080 Badajoz, Spain

Abbreviations: ADPGlc, ADP-glucose; ADPRib, ADP-ribose; ADPRibase, ADP-ribose pyrophosphatase; Ap_nA, diadenosine 5',5'''-P^1,P^n-*n*-phosphate; UDPGlc, UDP-glucose

2. MATERIALS AND METHODS

NAD^+ and NADH were from Boehringer and other nucleotide derivatives from Sigma. To remove contaminant ADPRib, NAD^+ was adsorbed onto Cl^- Dowex-1 and eluted with a 0-0.4 M NaCl linear gradient.

Except when indicated, enzyme activities were assayed by measuring P_i liberated by alkaline phosphatase with an ascorbate-molybdate reagent [13,14]. Standard assay mixtures contained, in 100 or 200 μl volume, 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 2.5 μg/ml alkaline phosphatase (Boehringer) and 500 μM substrate. Reactions were run at 37°C, stopped with 1.45 ml ascorbate-molybdate reagent [14], and A_{820} measured after 20 min at 45°C. ADPRibase was also assayed by coupling to alkaline phosphatase and adenosine deaminase, recording the decrease in A_{265} in a Shimadzu MSP 2000 spectrophotometer allowing for reliable measurement of 0.0002 *A* units/min, i.e. initial rates at below 1 μM ADPRib (ϵ for ADPRib-coupled hydrolysis, 8.6 $cm^{-1} \cdot mM^{-1}$). Standard conditions were 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 2.5 μg/ml alkaline phosphatase (Boehringer) and 1 μg/ml adenosine deaminase (Boehringer), at 37°C in 2-ml mixtures.

3. RESULTS

The isolation of ADPRibase-I and -II from rat

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

Table 1

Isolation of ADPRibase-I and ADPRibase-II from rat liver supernatants

	Volume (ml)	Protein (mg)	Activity (mU)	Specific activity (mU/mg)	Yield (%)
100 000 × *g* supernatant	16.5	310	950	3.1	100
$(NH_4)_2SO_4$ fractionation	4.5	120	580	4.8	61
Sephadex G-100 chromatography	33.5	44	650	14.8	68
DEAE-cellulose chromatography (ADPRibase-I)	35.0	8.8	330	37.5	35
DEAE-cellulose chromatography (ADPRibase-II)	35.0	1.1	210	191	22

10 g rat liver were homogenized in 20 ml buffer T [50 mM Tris-HCl (pH 7.5), 0.5 mM EDTA] and centrifuged for 60 min at 100 000 × *g*. Supernatant ADPRibase was fractionated successively by ammonium sulfate precipitation (30–60% saturation), gel filtration (fig.1A), and ion-exchange chromatography (fig.1B), upon which ADPRibase-I and ADPRibase-II were separated. Protein was assayed according to Bradford [15]

liver supernatants is summarized in table 1. In DEAE-cellulose elution where both pyrophosphatases were resolved, only one ADPGlc-hydrolase peak was found, coeluting with ADPRibase-II (fig.1B). Therefore, the phosphohydrolytic activities on NAD^+, NADH, Ap_2A and Ap_3A were also assayed in the ADPRibase peak fractions (fig.2). Besides ADPRib, only Ap_3A was split by fractions containing ADPRibase-I (fig.2A). Upon Cibacron blue F3G-A-Sepharose 4B chromatography, the contaminant activity (corresponding to dinucleoside triphosphatase, EC 3.6.1.29 [16]) bound to the gel, whereas ADPRibase-I appeared in the flow-through fraction (not shown). On the other hand, ADPRibase-II fractions showed low activity on NAD^+ or Ap_3A, but increasing activity on ADPGlc, Ap_2A, NADH and ADPRib with similar profiles (fig.2B).

The pyrophosphohydrolytic action of both ADPRibases was shown because they yielded AMP as a product detectable with AMP deaminase, whereas ADP or adenosine were not found with pyruvate kinase/lactate dehydrogenase or adenosine deaminase, respectively. The possibility that ADP is an ADPRibase product immediately converted to AMP by other enzymes seems to be ruled out since, when ADP substituted for ADP-Rib as the substrate, ADPRibase-I did not form AMP and ADPRibase-II did so at 20% of the rate found with the same ADPRib concentration.

ADPRibase-I showed a K_m value of 0.5 μM by initial rate assays (fig.3A) and by computing the

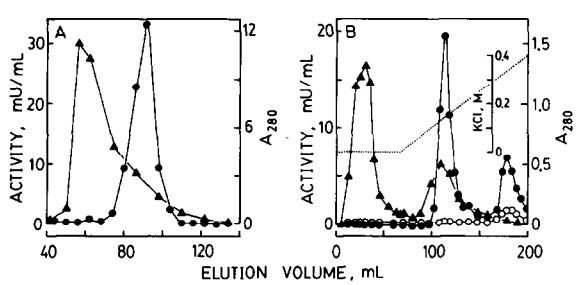

Fig.1. Separation of ADPRibase-I and -II. (A) 4 ml of the 30–60% ammonium sulfate fraction (table 1) were loaded onto a 1.2 × 103 cm Sephadex G-100 column equilibrated in and eluted with 50 mM Tris (pH 7.5), 0.5 mM EDTA (buffer T) at a rate of 20 ml/h. Fractions containing ADPRibase activity were pooled and (B) loaded onto a 2.2 × 6 cm DEAE-cellulose column equilibrated in buffer T and eluted at 30 ml/h with a linear 0–0.4 M KCl gradient in buffer T. (●) ADPRibase activity; (○) phosphohydrolytic activity on ADPGlc; (▲) A_{280}; (····) KCl. ADPRibase-I and -II are the peaks eluting in B at 100–135 and 165–200 ml, respectively.

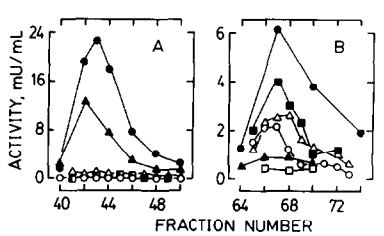

Fig.2. Substrate specificity of ADPRibase-I and -II. In an elution similar to that of fig.1B, the phosphohydrolytic activities on ADPRib (●), ADPGlc (○), NAD^+ (□), NADH (■), Ap_2A (△) and Ap_3A (▲) were measured with 500 μM substrate, except for the activities on Ap_3A and NAD^+, which were assayed with 260 and 70 μM substrate, respectively. NAD^+ was purified as described in section 2. Only the peak fractions corresponding to ADPRibase-I (A) or -II (B) were assayed. The activity on Ap_3A in A corresponds to an enzyme different from ADPRibase-I (see the text).

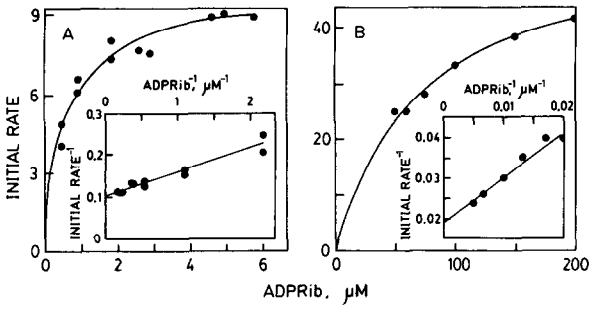

Fig.3. Saturation kinetics of ADPRibase-I (A) and -II (B). The initial ADPRib hydrolysis rates were determined by the adenosine deaminase and alkaline phosphatase-coupled assay. Double-reciprocal plots are shown in the insets. Rates are expressed as nmol ADPRib hydrolyzed/min per mg protein.

rate change along progression curves recorded until completion of the reaction (not shown). A spectrophotometric assay irreversibly coupled to alkaline phosphatase and adenosine deaminase was used (see section 2). Progression curves starting from 1 or 20 μM ADPRib yielded the same K_m values, indicating that potential inhibition by the expected products (inosine, ribose, P_i) did not interfere. The K_m value of ADPRibase-II for ADPRib was around 50 μM, 100-fold higher than that of ADPRibase-I (fig.3).

ADPRibase-I and -II did not work in the absence of divalent cations. With both enzymes, Mg^{2+}, Mn^{2+} and Ca^{2+} were tested and, at either 0.5 or 5 mM, Mg^{2+} supported full activity, Mn^{2+} supported 20% of the Mg^{2+}-dependent rate, and Ca^{2+} was inefficient. Both enzyme activities rose slightly with change in pH from 9 to 7. The ratios of activity at pH 7 to that at pH 9.2 were about 1.5.

4. DISCUSSION

ADPRibase-I is specific for ADPRib as substituting the ribose moiety by glucose or adding a second adenosine or a (reduced) nicotinamide residue to the ADPRib molecule prevented enzyme action. This and the low K_m value for ADPRib hydrolysis by ADPRibase-I support the occurrence of a turnover pathway specific for free ADPRib in liver.

ADPRibase-I differs from an activity described in rabbit reticulocytes, inhibited by Mg^{2+} or Mn^{2+} and of undefined substrate specificity [2], and from liver ADP-sugar pyrophosphatase (EC 3.6.1.21), that splits ADPGlc besides ADPRib [17], or phosphodiesterase I/nucleotide pyrophosphatase (EC 3.1.4.1/EC 3.6.1.9), which shows a wide substrate specificity [12,18]. A crude preparation of ADPRib pyrophosphatase (EC 3.6.1.13) from rabbit muscle [19], free of NAD^+/NADH pyrophosphatase, was not tested for activity with other substrates, precluding a comparison with ADPRibase-I, while its K_m for ADPRib was 38 μM, i.e. similar to the value displayed by ADPRibase-II (fig.3). ADPRibase-II was similar to ADPRibase-I as far as pH and Mg^{2+} or Mn^{2+} effects are concerned but the activity profiles in the DEAE-cellulose elution (figs 1B,2) indicated that ADPRibase-II, in contrast to ADPRibase-I, could be non-specific.

Acknowledgements: We are indebted to Drs Antonio Sillero and María Antonia Günther Sillero for encouragement and support. M.G.-D. was Alumno Interno por Oposición and Becario de Colaboración del MEC, affiliated to the Departamento de Bioquímica y Biología Molecular y Genética. This work was supported by grant 1159/84 from the CAICYT and grant PB87-1029 from the DGICYT.

REFERENCES

[1] Hayaishi, O. and Ueda, K. (1977) Annu. Rev. Biochem. 46, 95-116.
[2] Wu, J.M., Lennon, M.B. and Suhadolnik, R.J. (1978) Biochim. Biophys. Acta 520, 588-597.
[3] Ueda, K. and Hayaishi, O. (1985) Annu. Rev. Biochem. 54, 73-100.
[4] Tanuma, S., Kawashima, K. and Endo, H. (1986) J. Biol. Chem. 261, 965-969.
[5] Althaus, F.R. and Richter, C. (1987) ADP-Ribosylation of Proteins: Enzymology and Biological Significance, pp. 38-44, 66-92, Springer, Berlin.
[6] Wielckens, K., Schmidt, A., George, E., Bredehorst, R. and Hilz, H. (1982) J. Biol. Chem. 257, 12872-12877.
[7] Kun, E., Chang, A.C.Y., Sharma, M.L., Ferro, A.M. and Nitecki, D. (1976) Proc. Natl. Acad. Sci. USA 73, 3131-3135.
[8] Ueda, K., Kawaichi, M., Okayama, H. and Hayaishi, O. (1979) J. Biol. Chem. 254, 679-687.
[9] Richter, C., Winterhalter, K.H., Baumhuter, S., Lotscher, H.R. and Moser, B. (1983) Proc. Natl. Acad. Sci. USA 80, 3188-3192.
[10] Hilz, H., Koch, R., Fanick, W., Klapproth, K. and Adamietz, P. (1984) Proc. Natl. Acad. Sci. USA 81, 3929-3933.
[11] Ikejima, M. and Gill, D.M. (1985) Biochemistry 24, 5039-5044.
[12] Cameselle, J.C., Costas, M.J., Sillero, M.A.G. and Sillero, A. (1984) J. Biol. Chem. 259, 2879-2885.
[13] Ames, B.N. (1966) Methods Enzymol. 8, 115-118.

[14] Costas, M.J., Montero, J.M., Cameselle, J.C., Sillero, M.A.G. and Sillero, A. (1984) Int. J. Biochem. 16, 757–762.
[15] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[16] Sillero, M.A.G., Villalba, R., Moreno, A., Quintanilla, M., Lobatón, C.D. and Sillero, A. (1977) Eur. J. Biochem. 76, 331–337.
[17] Rodríguez, P., Bass, S.T. and Hansen, R.G. (1968) Biochim. Biophys. Acta 167, 199–201.
[18] Khorana, H.G. (1961) in: The Enzymes, 2nd edn (Boyer, P.D. et al. eds) vol. 5, pp. 79–94, Academic Press, New York.
[19] Doherty, M.D. and Morrison, J.F. (1962) Biochim. Biophys. Acta 65, 364–366.