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MICROCOCCUS RADIODURANS 5′-NUCLEOTIDASE

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SUMMARY

A 5′-nucleotidase (5′-ribonucleotide phosphohydrolase, EC 3.1.3.5) isolated from *Micrococcus radiodurans* appears to have properties more closely resembling some of the corresponding vertebrate enzymes than the reported bacterial enzymes. The *M. radiodurans* enzyme is a strict nucleoside 5′-phosphomonoesterase with a pH optimum between 8 and 9. It is maximally stimulated by Mg^{2+} but is active in the absence of added divalent cation, apparently possessing a bound metal ion. Deoxy-5′-nucleotides are the preferred substrates with purine nucleotides being hydrolyzed at a faster rate than the corresponding pyrimidine compounds. Two chemically altered nucleotides did not serve as substrates.

The enzyme is present in the particulate cell membrane fraction and can be released by detergent treatment. Its molecular weight was found to be 44 000 and its K_m for pdA was $8.1 \cdot 10^{-5}$ M.

INTRODUCTION

Bacterial 5′-nucleotidases (5′-ribonucleotide phosphohydrolase, EC 3.1.3.5) have been purified or partially purified from a number of organisms including *Escherichia coli*[1], *Salmonella typhimurium*, *Aerobacter aerogenes*, *Citrobacter*, *Shigello sonnei*[2], and *Proteus vulgaris*[3]. All the enzymes examined had a pH optimum for pA of about 6.0, were dependent on added metal ion, were optimally pppA and UDPG. stimulated by Co^{2+} and also hydrolyzed some pyrophosphate linkages such as ppA, A Fe^{2+} dependent 5′-nucleotidase has been described in *Clostridium sticklandii*[4] which has a broad neutral to alkaline pH optimum but this enzyme can also cleave phosphate from ppA and pppA.

This paper will describe a 5′-nucleotidase isolated from the radiation-resistant organism *Micrococcus radiodurans*, with properties considerably different from those described above.

Abbreviations: glx-pdG and glx-pG, the glyoxal adducts of pdG and pG, respectively.

MATERIALS AND METHODS

Sources of materials and supplies were as follows: Tryptone and yeast extract from Difco Laboratories; glucose, Triton X-100, and glyoxal (40% in water) from Baker Chemical Co.; sodium deoxycholate from Matheson Coleman and Bell; nucleosides and nucleotides from Calbiochem; lysozyme (EC 3.2.1.17) from Schwartz Bioresearch; pepsin (EC 3.4.4.1) and cytochrome *c* from Nutritional Biochemicals Corp; Sephadex G-25 and G-200 from Pharmacia Fine Chemicals; Whatman DEAE-cellulose DE52 from Mandel Scientific. All chemicals were reagent grade. The Diaflo concentration apparatus and membranes were obtained from the Amicon Corp., and the French pressure cell from the American Instrument Co. Pharmacia columns were used for column chromatography.

Thin-layer chromatography

Precoated thin-layer plates were run at room temperature in an Eastman Kodak apparatus. The systems used were as follows:

System a, cellulose plates (MN Polygram Cel 300; Mackerey, Nagel and Co.) run in saturated $(NH_4)_2SO_4$–100 mM sodium acetate (pH 6.0)–isopropanol (80:18:2, v/v/v).

System b, DEAE-cellulose plates (Bakerflex; J. T. Baker Chemical Co.) run in 25 mM Tris–HCl (pH 8.0).

Enzyme assays

The assays of 5′-nucleotidase activity described here measure the rate of release of P_i from pdA. To localize enzyme activity, as for instance in the effluent from a chromatographic column, the amount of P_i released after a single fixed time was measured. In addition to an appropriate amount of enzyme, the incubation mixture contained 5 mM pdA, 100 mM Tris–HCl (pH 8.0), and 10 mM $MgCl_2$ in a total volume of 0.2 ml. After 20 min at 30 °C, the reaction was stopped by the addition of 1.3 ml of water *plus* 1.5 ml of the molybdate reagent of Chen *et al.*[5]. Color was developed by incubation at 37 °C for 90 min and inorganic phosphate determined by measuring the absorbance at 815 nm.

The actual amount of activity present in a particular enzyme preparation was determined essentially as above except 0.2-ml aliquots were taken from a 2.0-ml incubation mixture at 10 min intervals for 1 h to ensure that the rate of P_i release with time was linear. 1 unit of activity is that amount of enzyme which will release 1 μmole of phosphorus in 1 h under the above conditions.

Up to and including Fraction 3 in the purification procedure the enzyme was added to the assay mixture in a solution containing 1.5% deoxycholate (w/v). Under all conditions used, the rate of release of phosphorus was proportional to enzyme concentration.

Specific activity is expressed as units of activity per unit of absorbance at 280 nm where the absorbance is measured as defined under Protein determination.

Protein determination

Protein was determined at different stages of the purification by measuring its absorbance at 280 nm in a 1-cm pathlength when dissolved in 10 mM Tris–HCl

(pH 8.0) containing 1.5% sodium deoxycholate (w/v). Absorbance was measured using a Beckman ACTA V Spectrophotometer.

Growth and preparation of cells

M. radiodurans was grown to stationary phase in liquid culture at 30 °C with vigorous aeration. The medium used was Tryptone, 5 g/l; yeast extract, 3 g/l; and glucose 1 g/l. The cells were harvested by centrifugation and were washed twice with 100 mM Tris–HCl (pH 8.0).

Synthesis of the glyoxal adduct of guanine nucleosides and nucleotides

5-mg samples of pG, G, pdG, and dG were each dissolved in 2 ml of 10 mM sodium acetate (pH 6.0) containing 3% glyoxal (v/v)[6,7]. The mixtures were incubated at 37 °C for 2 h and then lyophilized. Each sample was extracted twice at 0 °C with ethanol containing 10 mM $MgCl_2$. The remaining ethanol was removed under vacuum and the samples dissolved in 50 μl of water. 2.0 ml of the ice-cold ethanol–$MgCl_2$ solution was added to each and the mixtures kept in ice for 15 min. The samples were centrifuged at 50 000 × *g* for 15 min, washed with more ethanol–$MgCl_2$ solution and dried under vacuum. Examination by thin-layer chromatography in System a showed complete conversion to the glyoxal adduct in each case.

RESULTS

Enzyme purification

5 g of wet packed cells were suspended at 0 °C in 20 ml of 10 mM Tris–HCl (pH 8.0) saturated with *n*-butanol at 0 °C[8]. The cells were sedimented by centrifugation for 15 min at 50 000 × *g*, washed with 20 ml of ice-cold 100 mM Tris–HCl (pH 8.0) and recentrifuged.

Following resuspension in 5 ml of the above 100 mM Tris buffer, 10 mg of dry lysozyme was added and the mixture incubated at 37 °C for 1 h. Lysozyme and solubilized cell-wall material were removed from the incubation mixture by dilution with 20 ml of cold Tris buffer and centrifugation for 20 min at 50 000 × *g*. The pellet was washed in a further 20 ml of cold Tris buffer and sedimented as before.

The cells were lysed and the nucleotidase solubilized by suspension of the pellet for 30 min in 5 ml of an ice-cold 10% deoxycholate solution (w/v). The soluble material was separated by centrifugation for 15 min at 50 000 × *g*. The pellet was twice re-extracted with 5-ml portions of an ice-cold 5% deoxycholate solution (w/v) and then discarded.

Fraction 1. The combined supernatants were held overnight at 3 °C, centrifuged at 105 000 × *g* for 1 h and the pellet discarded.

Fraction 2. Fraction 1 was applied to a column of Sephadex G-200 and chromatographed as indicated in Fig. 1. The peak of activity was pooled as shown.

Fraction 3. Fraction 2 was concentrated to 11 ml by ultrafiltration at 0 °C in a Diaflo apparatus using an XM-50 membrane. Solid deoxycholate was then added to a concentration of 1.5% (w/v). The sample was chromatographed on a column of Sephadex G-200 containing deoxycholate as described in Fig. 2. The peak of activity was pooled as indicated.

Fraction 4. Fraction 3 was concentrated as before and centrifuged at 50 000 × *g*

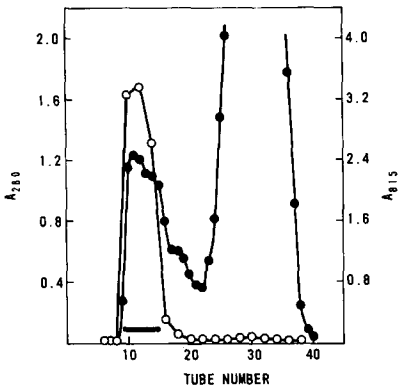

Fig. 1. Gel filtration on Sephadex G-200. The enzyme solution (13.6 ml; total $A_{280\ nm}$ 279; total activity 653 units) was applied to a Sephadex G-200 column (2.5 cm × 42 cm) equilibrated and eluted at room temperature with 10 mM Tris –HCl (pH 8.0). 8.5-ml fractions were collected at 40 ml/h. ●—●, $A_{280\ nm}$; ○—○, enzyme activity. The solid bar indicates the fractions pooled.

for 15 min. The supernate was desalted on a column of Sephadex G-25 (2.5 cm × 45 cm) equilibrated and eluted at 3 °C with 10 mM Tris–HCl (pH 8.0). The desalted preparation (19 ml) was made 1% in Triton X-100 (v/v) and applied to a column of DEAE-cellulose DE52 (0.9 cm × 12 cm) equilibrated and eluted at 3 °C with 10 mM Tris–HCl (pH 8.0) containing 1% Triton X-100. Protein which did not adhere to the column under these conditions was collected and concentrated as described above. Specific activity could not be measured at this stage because of the high absorption of Triton X-100 at 280 nm. The preparation was freed of Triton X-100 by chromatography on Sephadex G-200 under the conditions described in Fig. 2.

Even at this stage in the purification the enzyme required the presence of a detergent to prevent reaggregation. If Fraction 4 was reapplied to Sephadex G-200 under conditions as described in Fig. 1, it was no longer included in the gel and eluted with the column excluded volume.

For investigation of the properties of the enzyme, Fraction 4 was desalted on

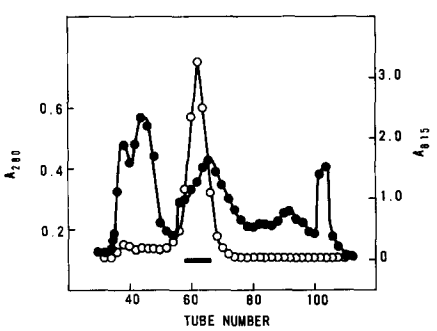

Fig. 2. Gel filtration on Sephadex G-200. The enzyme solution (11 ml, total $A_{280\ nm}$ 40.5, total activity 547 units) was applied to a Sephadex G-200 column (2.5 cm × 95 cm) equilibrated and eluted at room temperature with 10 mM Tris –HCl (pH 8.0) containing 1.5% deoxycholate (w/v). 5.0-ml fractions were collected at 40 ml/h. ●—●, $A_{280\ nm}$; ○—○, enzyme activity. The solid bar indicates the fractions pooled.

TABLE I

PURIFICATION OF *M. radiodurans* 5′-NUCLEOTIDASE

Fraction	*Total* $A_{280\ nm}$	*Total units of activity*	*Spec. act.*	*Yield (%)*
1. Deoxycholate extraction	279	653	2.34	100
2. Sephadex G-200 chromatography	40.5	547	13.5	84
3. Sephadex G-200 chromatography in deoxycholate	12.2	411	33.7	63
4. DEAE-cellulose chromatography in Triton X-100 *plus* rechromatography on Sephadex G-200 in deoxycholate	1.38	305	221	47

Sephadex G-25 as described above. The results of purification are summarized in Table I.

Cellular location of the 5′-nucleotidase

The particulate membrane fraction of the cell was prepared as follows: 1 g of wet packed cells was treated with *n*-butanol-saturated Tris–HCl (pH 8.0, 3.0 ml) and lysozyme (3 ml, 2 mg/ml) as described under Enzyme purification. The cells were then resuspended in 3.0 ml of 10 mM Tris–HCl (pH 8.0) and twice put through a French pressure cell. Microscopic examination of the solution showed no intact cells. This solution was centrifuged at 105 000 × *g* for 30 min. The sedimented red gelatinous membrane preparation was washed four times with 100 mM Tris–HCl (pH 8.0) and centrifuged each time at 105 000 × *g* for 30 min. The washed pellet was suspended in 3 ml of a 5% deoxycholate solution (w/v) and allowed to stand overnight at 3 °C and recentrifuged at 105 000 × *g* for 30 min. The final buffer wash and subsequent deoxycholate extract were assayed for 5′-nucleotidase activity. The last buffer wash released only 0.43 unit of nucleotidase into the supernatant. However, subsequent extraction of the membrane pellet with deoxycholate released 68 units of the enzyme. The total recovery of activity by this method is low due to incomplete sedimentation of the membranes fragmented into a gradient of sizes by the cell lysis procedure.

Acrylamide gel electrophoresis

As previously indicated, removal of detergent from the enzyme preparation allowed reaggregation. This aggregate does not enter acrylamide gels. Electrophoresis was therefore performed in acrylamide gels containing sodium dodecyl sulfate according to the procedure of Davis[9] as employed by Weber and Osborn[10].

The most highly purified enzyme preparations showed three bands of protein in the molecular weight range of 40 000 to 50 000. Since sodium dodecyl sulfate irreversibly inactivated the enzyme, it is not known which of these bands represented the 5′-nucleotidase.

Molecular weight determination

The purified nucleotidase from Fraction 4 was chromatographed on a Sephadex

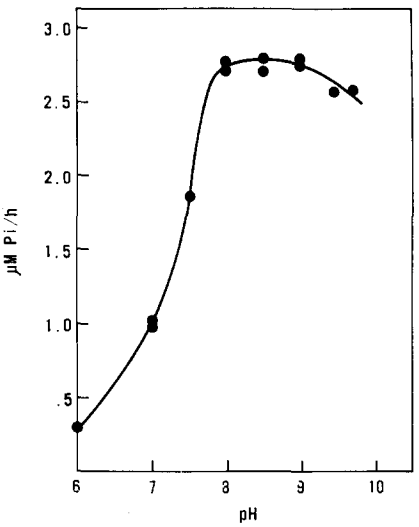

Fig. 3. pH curve of *M. radiodurans* 5′-nucleotidase. Assays of activity were performed as described under Enzyme assays except that the pH of the buffer was varied. At pH 6.0 sodium cacodylate was used in place of Tris–HCl.

G-200 column under the conditions described in Fig. 2. In addition three proteins of known molecular weight, bovine serum albumin[11], pepsin[12], and cytochrome *c*[13], were also run on the same column. The eluant volumes of these marker proteins were linearly related to the logarithms of their molecular weights. The molecular weight of the *M. radiodurans* 5′-nucleotidase determined by this method[14] was 44 000.

pH optimum and stability

The 5′-nucleotidase was assayed as indicated in Fig. 3. The optimum pH in 100 mM Tris–HCl was found to be between 8 and 9 with the enzyme becoming increasingly unstable as the pH increased above 9. At pH 8 in 100 mM Tris–HCl the enzyme gave zero order kinetics with respect to substrate for at least 90 min under the standard assay conditions. At pH 9 the rate was the same but linear kinetics could only be maintained for approximately one hour.

Up to and including Fraction 3 of the purification the enzyme appeared stable for at least two weeks when kept at 3 °C in the presence of either deoxycholate or Triton X-100. However, Fraction 4 lost all activity within two weeks at 3 °C whether or not either detergent was present.

Effect of various compounds on nucleotidase activity

The effect of deoxycholate on the rate of hydrolysis by 5′-nucleotidase was tested. The enzyme, dissolved in 10 mM Tris–HCl (pH 8.0) or the same buffer containing 1.5% deoxycholate (w/v), was added to the standard assay mixture and the rate of hydrolysis measured. Up to and including Fraction 3 in the purification procedure the enzyme preparation was visibly turbid or opalescent in the absence of deoxycholate and activity measurements indicated an apparent reduction in activity. The detergent appeared to cause a disaggregation of inhibiting proteins from the enzyme. However, the presence of deoxycholate had no effect on Fraction 4. At any

point in the purification 0.1% sodium dodecyl sulfate (w/v) used in place of deoxycholate under the above conditions completely eliminated activity.

5'-Nucleotidase activity was also measured by the standard assay procedure in the individual presence of the following compounds at the indicated final concentrations: 1 mM *p*-chloromercuribenzoate, 10 mM dithiothreitol, and 10 mM deoxyadenosine. The enzyme was added to the assay mixture containing the above compounds in two different ways: dissolved in 10 mM Tris–HCl (pH 8), and dissolved in the same buffer containing 1.5% deoxycholate (w/v). The effect of these compounds was also tested in the standard incubation mixture but with $MgCl_2$ omitted. No significant effect on hydrolysis rate was seen under any of the above conditions.

The effect of phosphate on the hydrolysis rate was measured by incorporating 10 mM potassium phosphate into the standard assay mixture. Aliquots were removed at intervals for 24 h and spotted for thin-layer chromatography in System a. No difference in rate of hydrolysis over a control not containing potassium phosphate could be detected.

Effect of divalent cations on 5'-nucleotidase

Various divalent cations were used in place of $MgCl_2$ in the standard assay mixture. The effect of EDTA in place of any divalent cation was also tested. All rates were linear with time except in the presence of EDTA where a slow drop in rate was

TABLE II

EFFECT OF DIVALENT CATIONS ON 5'-NUCLEOTIDASE

The indicated cations (all 10 mM and as the chloride) were substituted for $MgCl_2$ in 2.0 ml of the standard assay mixture. 0.2-ml samples were taken at 10-min intervals over 1 h for P_i determination and calculation of rate of hydrolysis, as described in Enzyme assays.

Divalent cation	*Relative rate*
Mg^{2+}	100
None	69
EDTA	57*
Ca^{2+}	84
Co^{2+}	48
Mn^{2+}	89
Cu^{2+}	13

* Non linear with time. Value given indicates approximate rate after 1 h.

seen over the entire 1 h hydrolysis. The presence of Cu^{2+} caused marked inhibition. The results are summarized in Table II. Mg^{2+} stimulation was a maximum at 10 mM and the effect of all other cations is shown at 10 mM. The other cations were also tested at lower and higher concentrations. No cation at any tested concentration was found to be more stimulatory than Mg^{2+} at 10 mM.

Substrate specificity

Phosphatase activity toward a number of compounds has been examined. The enzyme appears to be a strict nucleoside 5'-phosphomonoesterase. Nucleoside 2'- or 3'-phosphates are not hydrolyzed, nor are 5'-pyrophosphates. Relative rates of

TABLE III

ACTIVITY OF *M. radiodurans* 5′-NUCLEOTIDASE TOWARD VARIOUS NUCLEOTIDES

The incubation mixture (2 ml) contained 4 mg of nucleotide, 100 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$ and 0.06–0.12 units of enzyme activity. The mixture was incubated at 30 °C and 0.2-ml samples taken at 10-min intervals over 1 h for phosphate analysis as described in Enzyme assays.

Substrate	*Relative activity*
pdA	100
pdG	92
pdT	46
pdC	47
pA	50
pG	20
pU	14
pC	9
ppdA	0
pppdA	0
Ap (2′+3′)	0
Gp (2′+3′)	0
Cp (2′+3′)	0
Up (2′+3′)	0
ppA	0
pppA	0
UDPG	0

hydrolysis are shown in Table III. The products of all digestions were confirmed by thin-layer chromatography in Systems a and b.

ppdA, pppdA, ppA, pppA and Ap were also tested as potential competitive inhibitors of the hydrolysis of dpA. No inhibition was detected.

Activity of 5′-nucleotidase toward chemically modified substrates

The action of the 5′-nucleotidase on a substrate with a modified sugar was tested by the formation of borate adducts. Borate has the ability to form an adduct with the 2′ and 3′ vicinal hydroxyls of ribonucleotides[15]. This adduct cannot form on a deoxyribonucleotide.

The activity of the enzyme toward a substrate with a modified base was tested by formation of glyoxal adducts with guanine nucleotides. Glyoxal reacts specifically with guanine, of the four common bases and forms an additional 5-membered ring between N-1 and the amino group at Position 2 (ref. 6). This new ring has a pair of vicinal hydroxyls which can form a complex with borate in a manner analagous to the ribose hydroxyls[7].

The results are summarized in Table IV. Sufficient enzyme was added to the incubation mixture to completely hydrolyze pdG in 2.5 h in Tris buffer (pH 8.5). A control of pdA treated with glyoxal as described for guanine compounds showed complete conversion to dA under the digestion conditions.

Modification of the guanine nucleotide base with glyoxal completely inhibited the 5′-nucleotidase. The trace of hydrolysis seen after 20 h probably reflects the slight instability of the adduct at this pH[6,7].

The presence of borate buffer in place of Tris at the same pH results in a marked decrease in rate even when there are no vicinal hydroxyls to form a complex, as in the case of pdG. This inhibition was not relieved by increasing the concentration of

TABLE IV

ACTIVITY OF 5′-NUCLEOTIDASE TOWARD CHEMICALLY-MODIFIED SUBSTATES

The incubation mixture contained 2.5 mg/ml of nucleotide, 100 mM buffer, 10 mM $MgCl_2$ and was maintained at 23 °C. The products were identified and proportions estimated by thin-layer chromatography in System a.

Substrate	*Buffer*	*pH*	*Approx. % hydrolysis*	
			2.5 h	*20 h*
glx-pdG	Tris	7.5	0	trace
glx-pG	Tris	7.5	0	trace
pdG	Tris	7.5	95	100
pG	Tris	7.5	50	100
pdG	Borate	8.5	50	100
pdG	Tris	8.5	100	100
pG	Borate	8.5	0	20
pG	Tris	8.5	70	100
glx-pdG	Borate	8.5	0	0
glx-pG	Borate	8.5	0	0

Mg^{2+} to 200 mM (not shown in the table). However when the borate can form a complex with 2′, 3′ vicinal hydroxyls, as in pG, no digestion by 5′-nucleotidase could be seen after 2.5 h. The approximately 20% hydrolysis after 20 h may be a reflection of the equilibrium of the borate adduct.

Complexing glx-pdG with borate at pH 8.5 stabilized the glyoxal adduct at this pH[7] and completely prevented attack by the 5′-nucleotidase. The corresponding ribo compound glx-pG, which can bind borate to both the base and sugar, was likewise unhydrolyzed.

Determination of K_m

The K_m for this enzyme was determined using pdA, the substrate on which it it most active. The conditions of assay were similar to those described under Enzyme assays. The incubation mixtures contained 100 mM Tris-HCl (pH 8.0) and 10 mM

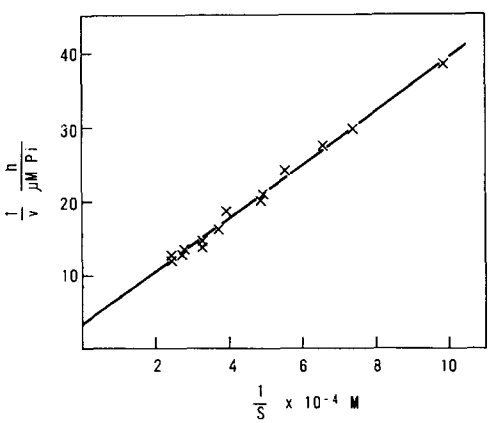

Fig. 4. Lineweaver–Burk plot of the hydrolysis of pdA by *M. radiodurans* 5′-nucleotidase. Enzyme assays were performed as described under Determination of K_m.

$MgCl_2$ in a total of 0.2 ml and were incubated at 30 °C for 15 min. 0.5 ml of molybdate reagent and 0.3 ml of water were added for the determination of released phosphorus. The Lineweaver–Burk plot (Fig. 4) is linear over the range of substrate concentrations examined. The K_m for pdA was found to be $8.1 \cdot 10^{-5}$ M.

DISCUSSION

The *M. radiodurans* 5′-nucleotidase appears to resemble some of the corresponding avian[16], mammalian[17–20] and reptilian enzymes[21,22] more closely than the reported bacterial enzymes[1–4]. Like the *M. radiodurans* enzyme, the vertebrate nucleotidases generally have been active in the absence of added metal ions, and Mg^{2+} or Mn^{2+} have usually been the most effective stimulators. In addition, these 5′-nucleotidases usually have alkaline pH optima and have activity only toward nucleoside 5′-phosphomonoesters. In contrast, the bacterial nucleotidases[1–4] and in particular the *E. coli* enzyme which has been best characterized[1], have acid or neutral pH optima, require added metal ion, often Ca^{2+} or Co^{2+}, and have other phosphatase activities, such as the ability to hydrolyze ppA, pppA and UDPG apparently present in the same protein.

The range of nucleotides hydrolyzed by *M. radiodurans* nucleotidase is quite small. The phosphate must be at the 5′ position and must not be part of a pyrophosphate linkage. Purine nucleotides are preferred with the corresponding pyrimidines being hydrolyzed at approximately half that rate. The presence of an additional 5-membered ring on the base portion of a purine nucleotide precludes activity. A 2′-hydroxyl group causes a 50–80% decrease in rate, and complexing vicinal 2′, 3′, hydroxyls with borate effectively blocks hydrolysis.

The distinct preference of the *M. radiodurans* nucleotidase for the deoxy-5′-nucleotides should be noted. This preference is in keeping with the special talent of this organism, that of being able to repair massive radiation damage to its DNA[23,24]. During repair of this damage, large amounts of DNA are degraded to 5′-mononucleotides[25]. Since the majority of the removed residues may be undamaged nucleotides[25,26], it would benefit the cell to be able to recover these residues as efficiently as possible. However, the inability of the 5′-nucleotidase to remove phosphate from an unnatural or damaged nucleotide would preclude reincorporation of these residues into the general nucleoside pool.

The action of EDTA on the *M. radiodurans* nucleotidase indicates a bound metal ion which is slowly removable by this chelating agent. The nature of this ion is unknown.

The presence of the *M. radiodurans* enzyme in the particulate membrane fraction after cellular lysis is consistent with the idea that bacterial nucleotidases are located in the periplasmic space[1,2] and may be bound to the cytoplasmic membrane. However this enzyme seems very firmly attached since lysozyme digestion failed to release it in significant quantities. The enzyme remained bound to this fraction during extensive washing and was only freed by treatment with a detergent. In the absence of detergent, there was a marked tendency for aggregation between the nucleotidase and other proteins. During the purification, advantage was taken of this effect by first chromatographing the enzyme on Sephadex G-200 in the absence of deoxycholate (Fig. 1), causing the aggregated enzyme to move with the column excluded volume.

Subsequent rechromatography on the same gel in the presence of detergent (Fig. 2) caused disaggregation and allowed the enzyme to move into the gel.

Other bacteria have been shown to contain an intracellular 5′-nucleotidase inhibitor protein[2]. Since the *M. radiodurans* enzyme preparation procedure involves cell lysis, any such inhibitor would likely have been bound to the 5′-nucleotidase. A purification step which removed the inhibitor would result in a net increase in total activity. No such increase was found. However, cell lysis in the presence of such an inhibitor may result in a portion of the 5′-nucleotidase being inhibited initially, not reactivated at any step, and simply removed during the enzyme isolation (especially since the inhibited enzyme would have a higher molecular weight and the purification included molecular sieving). This possibility cannot be eliminated since unlike *E. coli* and some other bacterial 5′-nucleotidases[1,2] the *M. radiodurans* enzyme is not solubilized by lysozyme digestion, osmotic shock or other techniques which do not release cytoplasmic proteins.

A mol. wt of 44 000 was obtained for the *M. radiodurans* 5′-nucleotidase. This compares closely with the value of 52 000 obtained for the *E. coli* enzyme by a similar method[1].

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