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A NOVEL PHOSPHODIESTERASE I FROM THE SOLUBLE FRACTION OF ERYTHROCYTES

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Summary

A previously unrecognized erythrocyte phosphodiesterase I with activity against thymidine-5′-monophospho-*p*-nitrophenyl ester is described. The enzyme is present in the soluble fraction of the erythrocyte, and was purified about 500-fold by chromatography using DEAE-cellulose, followed by gel chromatography with Sephadex G-200.

Erythrocyte phosphodiesterase I has a molecular weight of about 70 000, when fully active as a monomer. Its p*I* is 5.4 and the pH optimum is 8.5. The K_m value for thymidine-5′-monophospho-*p*-nitrophenyl ester is rather high, about 4 mmol/l. The enzyme has a barely detectable nucleotide pyrophosphatase activity. It is extremely sensitive to SH-inhibitors such as *N*-ethylmaleimide, *p*-chloromercuribenzoate and disulphides (a reversible 50% inhibition was obtained by cystamine, 0.01 mmol/l). It is a metalloenzyme with loosely bound metal, and is stimulated by Mg^{2+}. This activation by Mg^{2+} is counteracted by Zn^{2+}. Gel chromatography revealed that the enzyme is a monomer in the presence of Mg^{2+}. When inhibited by Zn^{2+}, it forms polymers that can be reconverted to the monomer by thiols.

All the above properties of the erythrocyte enzyme support the conclusion that it is different from plasma membrane phosphodiesterase I (oligonucleate 5′-nucleotidohydrolase, EC 3.1.4.1).

Introduction

During a study of the organ distribution of nucleotide pyrophosphatase (dinucleotide nucleotidohydrolase, EC 3.6.1.9) activity and phosphodiesterase I (EC 3.1.4.1) activity [1], we found a previously unrecognized phosphodi-

esterase I activity in erythrocytes. We speculated if the enzyme might be a remnant from erythrocyte precursor cells, involved in a nucleic acid degradation pathway. The present study deals with this enzyme, and shows that phosphodiesterase I present in erythrocytes has different properties from phosphodiesterase I, previously studied in different mammalian tissues.

Materials and Methods

Chemicals. Thymidine-5′-monophospho-*p*-nitrophenyl ester, thymidine-3′-monophospho-*p*-nitrophenyl ester, 4-nitrophenyl phenylphosphonate, bis(4-nitrophenyl)phosphate, glycerophosphocholine, 5-nucleotidase (from *Crotalus Atrox* venom) and 3′,5′-cyclic nucleotide phosphodiesterase (from bovine heart) were obtained from Sigma Chemical Company, St. Louis, MO, U.S.A. Alkaline phosphatase (from calf intestine) was obtained from Boehringer Mannheim GmbH, Mannheim, F.R.G. As reference compounds for the estimation of molecular weight of the enzyme by gel filtration we used a kit (MS II) from Serva Fein-biochemica, Heidelberg, F.R.G. Ultrodex and Ampholine for electrofocusing were obtained from LKB-Produkter AB, S-16125, Bromma, Sweden. Diethylaminoethyl cellulose (DE-52) was obtained from Whatman Ltd., Springfield Mill, Maidstone, Kent, U.K. Sephadex G-200 and activated thiol-Sepharose 4B was obtained from Pharmacia Fine Chemicals AB, Box 175, S-75104 Uppsala 1, Sweden. Uridine diphosphate glucose uniformly labelled in the glucose moiety with ^{14}C, 233.5 Ci/mol, was obtained from New England Nuclear, Boston, MA, U.S.A. Other chemicals were products of analytical grade.

Assay of phosphodiesterase I activity. When phosphodiesterase activity was estimated in materials free of hemoglobin, thymidine-5′-monophospho-*p*-nitrophenyl ester was incubated at 37°C with the material to be examined in a total volume of 500 μl in Tris-HCl buffer (0.3 ml/l, pH 8.5) with or without the addition of Mg^{2+} and/or dithiothreitol. To obtain maximal stimulation we added Mg^{2+} (1 mmol/l) and dithiothreitol (10 mmol/l). In some of the experiments we used only Mg^{2+} (1 mmol/l) or dithiothreitol (0.5 mmol/l) as stimulator depending on the purpose of the experiment. The reaction was stopped by addition of 250 μl NaOH (0.5 mol/l), and 750 μl water were added before readings at 405 nm. Reagent blanks were run in parallel and the enzyme-containing material was added after NaOH. The enzyme activity was calculated from a mmol extinction coefficient for the product *p*-nitrophenol of 17.6 $l \cdot mmol^{-1} \cdot cm^{-1}$ [2]. When the activity in crude erythrocyte lysate was determined, the reaction was stopped and hemoglobin was precipitated by adding 250 μl $HClO_2$ (2 mol/l) followed by alkalinization/precipitation of the perchlorate with KOH.

Assays of other enzymes. Nucleotide pyrophosphatase (EC 3.6.1.9) was estimated as described previously [1,3]. Alkaline phosphatase activity was estimated by a method recommended by the Committee on Enzymes of The Scandinavian Society for Clinical Chemistry and Clinical Physiology [4]. Glycerophosphoryl cholinesterase activity was estimated by the method of Fowler and de Duve [5]. Cyclic 3′,5′-nucleotide phosphodiesterase activity was measured by the method of Butcher and Sutherland [6] with one modification: we used

alkaline phosphatase instead of 5-nucleotidase for the second reaction (hydrolysis of the 5′-phosphate group). When 4-nitrophenyl phenylphosphonate, bis(4-nitrophenyl)phosphate or thymidine-3′-monophospho-*p*-nitrophenyl ester was used as the substrate, the other assay conditions were as those used when estimating phosphodiesterase activity with thymidine 5′-monophospho-*p*-nitrophenyl ester.

Preparation of hemolysate. Bank blood was used in most experiments, since the same results were obtained with this material and with freshly-drawn heparin blood. When preparing the hemolysate we followed the method of Hatfield and Wyngaarden [7] with one modification: we obtained lysis of the cells by freeze-thawing.

Procedure for the partial purification of erythrocyte phosphodiesterase I. As the first step in the purification procedure, the erythrocyte lysate was centrifuged at 12 100 × *g* for 30 min. The supernatant was subjected to ion-exchange chromatography with DEAE-cellulose as described by Hatfield and Wyngaarden [7]. Using this procedure we isolated a hemoglobin-free eluate and for further purification we used gel filtration on a Sephadex G-200 column (dimensions: 2.5 × 40 cm, i.e., bed vol. approx. 200 ml). The column was equilibrated with Tris-HCl buffer (0.05 mol/l, pH 7.5)/KCl (0.1 mol/l)/glycerol (10%, v/v). Glycerol was added because we found that glycerol counteracted loss of enzyme activity during the purification procedures. (Dimethyl sulfoxide and thymidine-5′-monophospho-*p*-nitrophenyl ester also protected the enzyme, but less efficiently.) The same solution was used as eluant. The gel filtrations were performed at 4°C. About 2.5 ml of the sample were introduced into the column from the bottom, using a peristaltic pump. The flow of the eluant was 20 ml/h. Column effluents were collected in 3 ml fractions from the top of the column.

Estimation of molecular weight by gel filtration. Estimation of molecular weight of phosphodiesterase I from erythrocytes was carried out by gel filtration according to Andrews [8], using the same column and the same eluant as described for the purification procedure. The reference proteins were cytochrome *c* (M_r 12 400), chymotrypsin A (25 000), albumin (67 000) and catalase (240 000).

Determination of isoelectric point. The isoelectric point of phosphodiesterase I in erythrocytes was determined by flat bed electrofocusing in a granulated gel with LKB 2117 Multiphore, according to LKB Application Note 198 (LKB-Produkter AB, S-16125, Bromma, Sweden) with some modifications. Instead of Sephadex G-75 superfine we used Ultrodex. Aliquots of the different zones were removed for the estimation of pH [9] and the activity of phosphodiesterase I. The ampholytes were removed by gel chromatography on Sephadex G-25 Medium.

Results

Table I shows the different steps in the partial purification of human erythrocyte phosphodiesterase I. The activity is confined to the soluble fraction of the erythrocytes. By chromatography on DEAE-cellulose (to remove hemoglobin), a purification of about 100-times was obtained. An additional purifica-

TABLE I

PURIFICATION OF ERYTHROCYTE PHOSPHODIESTERASE I

Incubations were performed in Tris-HCl buffer (0.3 mol/l, pH 8.5). Incubation time: 20 min. Substrate: Thymidine-5-monophospho-*p*-nitrophenyl ester (10 mmol/l). Additions: Mg^{2+} (1 mmol/l) and dithiothreitol (10 mmol/l). Glycerol (10%, v/v) was present during gel chromatography.

Purification step no.	Fraction	Spec. act. (μmol/g protein per min)	Amount of protein (mg) in the fraction	Times of purification
1	Crude erythrocyte lysate	0.44	590.00	—
2	Erythrocyte soluble fraction	0.46	592.00	1.05
3	2.55 ml pooled eluate from chromatography on DEAE-cellulose	46.22	39.98	105
4	Fraction no. 37 from chromatography on Sephadex G-200	235.29	0.51	535

tion by a factor of 5 was obtained by the next step using gel chromatography with Sephadex G-200.

Separation of the enzyme protein from other proteins in the Sephadex G-200 eluate was obtained by covalent chromatography with activated thiol-Sepharose 4B, but with considerable loss of enzyme activity (even in the presence of glycerol, see below). By flat bed isoelectric focusing of eluate from DEAE-cellulose chromatography we found erythrocyte phosphodiesterase I activity in two protein bands, at pH 5.37 and 5.42, respectively, but the enzyme activity was also strongly reduced by this procedure. Several additions were tested for their ability to counteract loss of enzyme activity during purification. Glycerol (10%, v/v) improved recovery during gel chromatography, but we could not obtain higher than about 500-times purification (Table I). In experiments for characterization of the enzyme, we mainly used the material with highest specific activity, i.e., that obtained by chromatography using DEAE-cellulose followed by chromatography with Sephadex G-200.

Using gel chromatography, the molecular weight of erythrocyte phosphodiesterase I was estimated to be slightly below 70 000 (data not shown).

The enzyme in crude lysate and in all steps of purification has a rather sharp pH optimum of about 8.5 (data not shown), whereas plasma membrane phosphodiesterase I (EC 3.1.4.1) has a pH optimum of 9.8 [3].

Table II shows that erythrocyte phosphodiesterase I was less active with bis-(4-nitrophenyl)phosphate and 4-nitrophenyl phenylphosphonate than with thymidine-5′-monophospho-*p*-nitrophenyl ester, and that it had no or barely detectable activity with thymidine 3′-monophospho-*p*-nitrophenyl ester, glyceryl phosphorylcholine, cyclic 3′,5′-AMP or UDP-glucose. Table II also shows

TABLE II

ACTIVITY OF HUMAN ERYTHROCYTE PHOSPHODIESTERASE I WITH DIFFERENT SUBSTRATES

Enzyme	Substrate	Activity per (μmol/g protein per min)
Erythrocyte phosphodiesterase I, partially purified with DEAE-cellulose and Sephadex G-200	Thymidine-5′-monophospho-*p*-nitrophenyl ester	142
	bis(4-nitrophenyl) phosphate	8
	Thymidine-3′-monophospho-*p*-nitrophenyl ester	0
	Glyceryl phosphoryl choline	0
	cyclic 3′,5′-AMP	Trace
	4-nitrophenyl-phenyl-phosphonate	27
	UDP-glucose	Trace
Commercial alkaline phosphatases from calf intestine	Thymidine-5′-monophospho-*p*-nitrophenyl ester	0
Commercial-5′-nucleotidase from *Crotalus atrox* venom	Thymidine-5′-monophospho-*p*-nitrophenyl ester	Trace
Cyclic 3′,5′-nucleotide phosphodiesterase from bovine heart	Thymidine-5′-monophospho-*p*-nitrophenyl ester	0

that thymidine-5′-monophospho-*p*-nitrophenyl ester is not a substrate for commercial alkaline phosphatases, 5′-nucleotidase or cyclic 3′,5′-nucleotide phosphodiesterase.

Table III shows the effects of some divalent cations, nucleotides and other additions on erythrocyte phosphodiesterase I in crude erythrocyte lysate and a partially purified enzyme preparation. The enzyme was stimulated by Mg^{2+}, and less efficiently by Mn^{2+} in the partially purified fraction. Probably, endogenous dication(s) is removed in the purification procedure. Ni^{2+}, and particularly Zn^{2+} and Cu^{2+} inhibit the enzyme activity strongly. EDTA also blocks the enzyme activity. ATP inhibits the enzyme moderately, particularly in the most purified fraction, possibly because of its chelating properties. UDP-glucose did not inhibit the enzyme significantly. As found previously [1], the enzyme activity was stimulated by thiols.

Table IV shows that dialysis against NaCl (0.15 mol/l) caused a pronounced decrease of the activity of phosphodiesterase I in erythrocytes, but not in serum or liver homogenate. The erythrocyte phosphodiesterase I activity could be restored by the addition of Mg^{2+}. Dialysis against EDTA (5 mmol/l) almost removed phosphodiesterase I activity in all fractions tested. The activity in erythrocytes could be completely restored by the addition of Mg^{2+} (and the liver membrane enzyme activity could be partially restored by the addition of Zn^{2+}).

TABLE III

EFFECTS OF DIVALENT CATIONS, NUCLEOTIDES, EDTA AND THIOLS ON THE ACTIVITY OF CRUDE AND PARTIALLY PURIFIED HUMAN ERYTHROCYTE PHOSPHODIESTERASE I

Incubations were performed in Tris-HCl buffer (0.3 mol/l, pH 8.5). Incubation time: 60 min. Substrate: thymidine-5'-monophospho-*p*-nitrophenyl ester (10 mmol/l). Amounts of protein in assay with crude erythrocyte lysate: 26 mg, with eluate from chromatography using DEAE-cellulose: 0.24 mg. Activity without additions: crude lysate 330 nmol/g protein per min; partially purified by chromatography with DEAE-cellulose 2029 nmol/g protein per min.

Additions	Concentration (mmol/l)	Activity (% of control without additions)	
		Crude erythrocyte lysate	Enzyme preparation partially purified by chromatography with DEAE-cellulose
None	—	100	100
Ca^{2+}	1.0	63	61
Mg^{2+}	1.0	131	1329
Mn^{2+}	1.0	73	761
Zn^{2+}	1.0	3	0
Ba^{2+}	1.0	—	29
Ni^{2+}	1.0	47	23
Cu^{2+}	1.0	0	0
EDTA	1.0	1	0
5'AMP	2.0	89	99
UDP-glucose	0.4	104	100
ATP	0.4	70	91
l'Cysteine	10.0	126	127
Dithiothreitol	10.0	158	229

Fig. 1 shows that human erythrocyte phosphodiesterase is strongly inhibited by Zn^{2+}. A Zn^{2+} concentration of 0.001 mmol/l caused a reduction of the activity to 40%. Fig. 1 also shows that human erythrocyte phosphodiesterase I is strongly inhibited by cystamine. 50% inhibition was present at 0.01 mmol/l, and close to 100% inhibition was evident at 1 mmol/l. The enzyme was also strongly inhibited by other disulphides. Thus, about 50% inhibition was obtained by 0.01 mmol/l tetrathionate, about 0.2 mmol/l cystine and 1 mmol/l glutathione disulphide (data not shown). The inhibition by cystamine (0.1 mmol/l) could be completely reversed by thiols (e.g., cysteamine 5 mmol/l and dithiothreitol 5 mmol/l). Erythrocyte phosphodiesterase was almost completely inhibited also by *p*-chloromercuribenzoate (0.01 mmol/l) or *N*-ethylmaleimide (1 mmol/l). Also the inhibition by *p*-chloromercuribenzoate could be reversed by dithiothreitol.

Fig. 2. shows the erythrocyte phosphodiesterase I activity with different concentrations of Mg^{2+} with and without addition of Zn^{2+}. The experiment was carried out in the presence of dithiothreitol (0.5 mmol/l) as stimulating agent. Because of the ability of dithiothreitol to chelate Zn^{2+} (see below), the concentration of Zn^{2+} in this experiment had to be as high as 0.1 mmol/l to obtain an appropriate inhibition. Half-maximal activity was obtained at a Mg^{2+} concentration of about 0.04 mmol/l. Fig. 2 also shows that the inhibition by Zn^{2+} is incompletely counteracted by the addition of Mg^{2+}. In Lineweaver-Burk plots of such experiments (data not shown), both the K_m for Mg^{2+} and V are altered

TABLE IV

EFFECT OF DIALYSIS AGAINST NaCl OR EDTA ON PHOSPHODIESTERASE I FROM ERYTHROCYTES AND OTHER SOURCES

Dialysis was performed overnight at 4° C.

Additions	Conc. (mmol/l)	Undialyzed			Dialyzed against NaCl (0.15 mol/l)			Dialyzed against EDTA (5 mmol/l)		
		Serum (nmol/g protein per min)	Erythrocytes (nmol/g protein per min)	Liver (μmol/g protein per min)	Serum (nmol/g protein per min)	Erythrocytes (nmol/g protein per min)	Liver (μmol/g protein per min)	Serum (nmol/g protein per min)	Erythrocytes (nmol/g protein per min)	Liver (μmol/g protein per min)
None	—	330	315	127	332	69	119	2	4	9
Mg^{2+}	1	339	412	132	340	459	126	2	476	15
Zn^{2+}	1	327	9	124	327	16	117	19	6	50

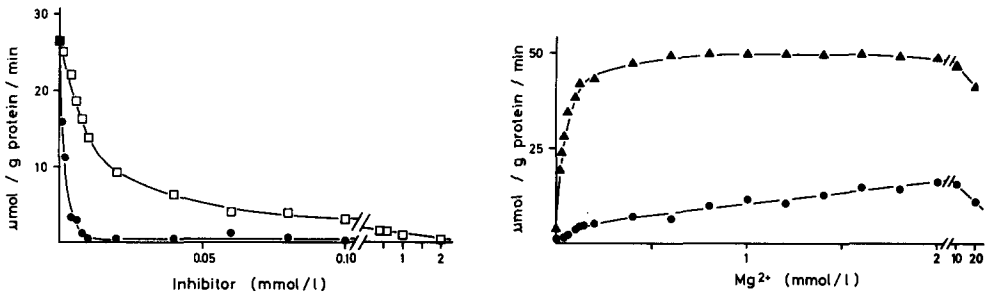

Fig. 1. Inhibition of human erythrocyte phosphodiesterase I by cystamine or Zn^{2+}. Erythrocyte phosphodiesterase I (0.018 mg protein) partially purified by ion-exchange chromatography, using DEAE-cellulose followed by gel filtration with Sephadex G-200, was incubated for 60 min at 37° C with thymidine-5′-monophospho-*p*-nitrophenyl ester (10 mmol/l) and Mg^{2+} (1 mmol/l) in Tris-HCl buffer (0.3 mol/l, pH 8.5). •, with Zn^{2+} □, with cystamine (also present in a preincubation without substrate at 37° C, pH 8.5 for 10 min).

Fig. 2. Effect of adding Mg^{2+} on the activity of human erythrocyte phosphodiesterase I with and without Zn^{2+} present. Erythrocyte phosphodiesterase I (0.02 mg protein) partially purified by the same procedures as stated in legend to Fig. 1 was incubated for 60 min at 37° C with thymidine-5′-monophospho-*p*-nitrophenyl ester (10 mmol/l) and dithiothreitol (0.5 mmol/l) in Tris-HCl buffer (0.3 mol/l, pH 8.5). •, with Zn^{2+} (0.1 mmol/l). ▲, without Zn^{2+}.

when Zn^{2+} is added, i.e., the plots fit with a 'mixed type' mechanism. Probably, part of the Zn inhibition is due to competition with Mg^{2+} for a metal binding site on the enzyme.

Fig. 3. shows a double-reciprocal plot of the activity of erythrocyte phos-

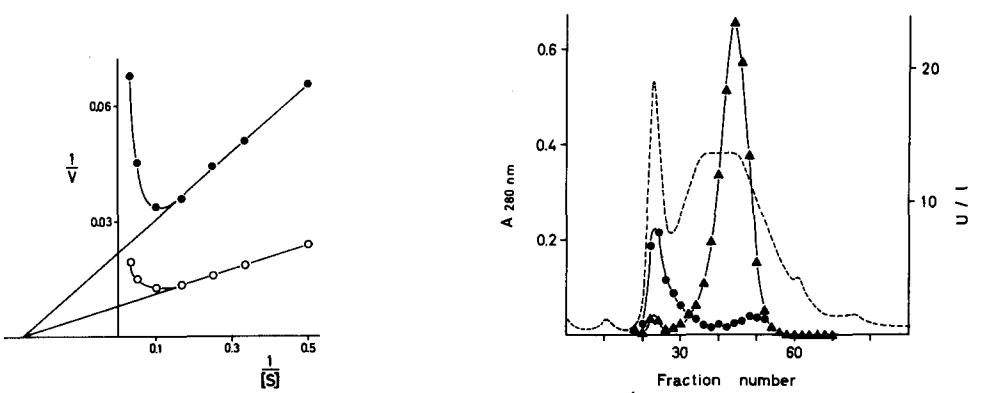

Fig. 3. Double-reciprocal plot showing substrate inhibition by 5′-monophospho-*p*-nitrophenyl ester of partially purified human phosphodiesterase I and the effect of adding Zn^{2+}. Erythrocyte phosphodiesterase I (0.019 mg protein) was incubated for 60 min at 37° C with thymidine 5′-monophospho-*p*-nitrophenyl ester (10 mmol/l) and dithiothreitol (0.5 mmol/l) in Tris-HCl buffer (0.3 mol/l, pH 8.5). *V* is expressed as µmol/g protein per min, and [S] expresses 0.5 · (initial + final) conc. (mmol/l). •, with Zn^{2+} (0.1 mmol/l). ○, without Zn^{2+}.

Fig. 4. Gel chromatography with Sephadex G-200 of Mg^{2+}-stimulated or Zn^{2+}-inhibited human erythrocyte phosphodiesterase I. Human erythrocyte phosphodiesterase I, partially purified by chromatography using DEAE-cellulose, was preincubated with $MgCl_2$ (5 mmol/l) or $ZnCl_2$ (0.05 mmol/l) in Tris-HCl buffer (0.025 mol/l; pH 7.5) and KCl (0.05 mol/l) for 5 min, and passed through a Sephadex G-200 column with Tris-HCl buffer (0.05 mol/l; pH 7.5)/KCl (0.1 mol/l)/$MgCl_2$ (10 mmol/l) or $ZnCl_2$ (0.1 mmol/l) as eluant. The ordinates show the amount of protein by A_{280nm} in the experiment with Mg^{2+} (stippled curve), or the enzyme activity with Mg^{2+} (▲) or Zn^{2+} (•), respectively.

phodiesterase I with thymidine-5′-monophospho-*p*-nitrophenyl ester in the presence of dithiothreitol (0.5 mmol/l) with and without the addition of Zn^{2+}. A pronounced substrate inhibition was found. The K_m value appears to be high, about 4 mmol/l. When Zn^{2+} (0.1 mmol/l) was added, the K_m value was unaltered (i.e., the inhibition is non-competitive). Probably, Zn^{2+} does not compete for the substrate-binding site of the enzyme.

Dithiothreitol reversed the inhibitory effects of Zn^{2+} on erythrocyte phosphodiesterase I. Attempts were made to reveal if the mechanism of this reversal might be chelation of the metal. In uncited experiments, EDTA counteracted the Zn-inhibition of phosphodiesterase I at equimolar concentrations. Further, EDTA and dithiothreitol both counteracted the effect (stimulation) of Zn^{2+} on dialyzed, purified alkaline phosphatase from calf intestine. Both agents are thought to exert this effect by chelating Zn^{2+}. However, the concentration of dithiothreitol had to be 100-times that of EDTA to obtain the same effect. Even though dithiothreitol was thus shown to chelate Zn^{2+} rather weakly, this mechanism may at least in part explain also its reversal of the Zn-inhibition of erythrocyte phosphodiesterase I. Additional effects of dithiothreitol on SH-group(s) of the enzyme cannot be excluded, however.

Fig. 4 shows that by gel chromatography, it could be demonstrated that erythrocyte phosphodiesterase I in the presence of Mg^{2+} was present as the monomer. Less than 5% of the activity was present as a species of higher molecular weight. In the presence of Zn^{2+} (when the enzyme activity was strongly inhibited), the enzyme was essentially present as polymer, with only a minor fraction as monomer. When dithiothreitol (1 mmol/l) was present in addition to Zn^{2+} (0.01 mmol/l), the enzyme was reconverted to the monomeric form, and its activity was about half restored.

Since erythrocyte phosphodiesterase I might be derived from nuclei (or nuclear remnants), its activity during induction (by bleeding) of experimental reticulocytosis in rabbits was examined. No significant increase of the enzyme activity was found when the reticulocyte count increased from 23 to 119‰. Nucleus-containing (fowl) erythrocytes had enzyme activities at the same level as mammalian erythrocytes (results not shown).

Discussion

In the present study, investigations on a novel phosphodiesterase I from erythrocytes [1] is presented. This enzyme is different from phosphodiesterase I (EC 3.1.4.1) which is present in the plasma membrane of cells from different organs [1,10—12]. The latter enzyme has been purified from rat liver plasma membranes [12]. Thus, the erythrocytes enzyme is present in the soluble fraction, whereas the previously known enzyme is membrane bound. Its molecular weight is 70 000, in contrast with 137 000 for the plasma membrane enzyme [13]. The p*I* value of the erythrocyte enzyme is 5.4, whereas the plasma membrane enzyme has a p*I* value of about 4.5 [14]. The pH optimum of erythrocyte phosphodiesterase is lower, 8.5. vs. 9.8. Its K_m for thymidine-5′-monophospho-*p*-nitrophenyl ester is higher, 4 vs. 0.25 mmol/l. Also, the erythrocyte enzyme shows only a barely detectable activity with UDP-glucose as substrate, in contrast with phosphodiesterase I (EC 3.1.4.1), which has considerable

nucleotide pyrophosphate activity. The erythrocyte enzyme is stimulated by Mg^{2+}, and inhibited by Zn^{2+}. The plasma membrane enzyme, on the other hand, is activated by Zn^{2+}, and much less by Mg^{2+} [1]. The activating metal is much more loosely bound in the erythrocyte enzyme than in the plasma membrane phosphodiesterase I. The erythrocyte enzyme is very sensitive to inhibition by disulphides, in contrast with the plasma membrane enzyme, which on the contrary is inhibited by thiols [1]. The erythrocyte enzyme also has a remarkable tendency to form polymer series. The conclusion that the erythrocyte phosphodiesterase I described in the present communication is different from phosphodiesterase I from plasma membranes (EC 3.1.4.1) seems thus sufficiently documented.

The activity of erythrocyte phosphodiesterase I is completely dependent on the presence of divalent cations, and with Mg^{2+}, it shows half-maximal activity (K_m) at about 0.04 mmol/l. Mn^{2+} also stimulates the enzyme, but other cations studied do not.

The erythrocyte enzyme is strongly inhibited by Zn^{2+}. This inhibition appears to be partly due to competition for a metal binding site, since the inhibition by Zn^{2+} is reversed by Mg^{2+} (although incompletely). The mechanism of this inhibition is of a 'mixed type'. This type of inhibition is present whether dithiothreitol is added at concentrations that will partially reverse the Zn^{2+} inhibition, or not. Zn^{2+} is not competitive with the substrate, however. The most active molecular species of the enzyme is the monomer which is formed in the presence of Mg^{2+}. However, the inhibition by Zn^{2+} appears to occur by more than one mechanism (see below) since it is only partially counteracted by Mg^{2+}.

The other mechanism is probably an inhibition of SH-group(s). Erythrocyte phosphodiesterase I is namely an enzyme with an extremely reactive SH-group, a property that is revealed by a 50% loss of activity when cystamine is added at a concentration as low as 0.01 mmol/l. Its sensitivity towards disulphides is thus comparable to that of papain [15], but far greater than that of hexokinase [16]. Erythrocyte phosphodiesterase was also inhibited by *N*-ethylmaleimide or *p*-chloromercuribenzoate at low concentration, when the conditions were chosen for reactions specificially with the SH-group [17]. The inhibition by disulphides is easily reversed by consecutive addition of thiols. An effect on the SH-group may even explain part of the inhibition by Zn^{2+} of erythrocyte phosphodiesterase I. The addition of Zn^{2+} was followed by inhibition and extensive polymerization of the enzyme. Both effects were counteracted by the addition of a thiol such as dithiothreitol. This reversal is partially explained by chelating properties of dithiothreitol, even though this thiol is a weak chelator of Zn^{2+} as compared with EDTA.

The present study has not given any clue to the metabolic role of this enzyme. Its activity is rather low, and its K_m for the most efficient substrate, thymidine-5′-phospho-*p*-nitrophenyl ester, is high. The enzyme is less active with bisnitrophenyl phosphate, and is without activity with 3-phosphate ribose compounds or with nucleotide pyrophosphates. The specific activity of erythrocyte phosphodiesterase I is not higher in nucleus containing erythrocytes or in reticulocytes. This may suggest that it is not a remnant from a nucleic acid degradation pathway present in erythrocyte precursors.

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