Biochimica et Biophysica Acta, 525 (1978) 346—356
© Elsevier/North-Holland Biomedical Press

BBA 68496

TWO PURINE NUCLEOSIDE PHOSPHORYLASES IN *BACILLUS SUBTILIS*

PURIFICATION AND SOME PROPERTIES OF THE ADENOSINE-SPECIFIC PHOSPHORYLASE

KAJ FRANK JENSEN

Enzyme Division, University Institute of Biological Chemistry B, Sølvgade 83, DK-1307 Copenhagen (Denmark)

(Received February 9th, 1978)

Summary

Two purine nucleoside phosphorylases (purine-nucleoside:orthophosphate ribosyltransferase, EC 2.4.2.1) were purified from vegetative *Bacillus subtilis* cells.

One enzyme, inosine-guanosine phosphorylase, showed great similarity to the homologous enzyme of *Bacillus cereus*. It appeared to be a tetramer of molecular weight 95 000.

The other enzyme, adenosine phosphorylase, was specific for adenosine and deoxyadenosine. The molecular weight of the native enzyme was 153 000 ± 10% and the molecular weight of the subunits was 25 500 ± 5%. This indicates a hexameric structure. The adenosine phosphorylase was inactivated by 10^{-3} M *p*-chloromercuribenzoate and protected against this inactivation by phosphate, adenosine and ribose 1-phosphate.

Introduction

Purine nucleoside phosphorylase (purine-nucleoside:orthophosphate ribosyltransferase, EC 2.4.2.1) catalyzes the reaction:

Purine-(deoxy)nucleoside + phosphate ⇌ Purine + (deoxy)ribose 1-phosphate

The enzymes from a variety of biological sources have been investigated [1,2] and considerable variation in their properties has been reported.

The variations include differences in specificity, particularly in the ability to phosphorylyse adenine nucleosides. The enzymes from enteric bacteria [3,4] and mycoplasma [5] split adenine nucleosides as well as they split hypoxanthine and guanine nucleosides. In contrast, adenine nucleosides are very poor substrates for enzymes from most other sources [1,2].

Recently, a partial physical separation of the adenosine phosphorolytic activity from the inosine-guanosine phosphorolytic activity of extracts of vegetative

Bacillus subtilis cells was reported for the first time [6]. This shows the existence in *B. subtilis* of a phorphorylase specific for adenine nucleosides, in addition to the known purine nucleoside phosphorylase (which will phosphorolyse hypoxanthine and guanine nucleosides). This latter enzyme has previously been characterized from *Bacillus cereus* [7–9].

Since we are studying the nucleoside salvage pathways of *B. subtilis*, we were interested in knowing the properties of this new adenosine phosphorylase. No similar enzyme has been found in other organisms. This report describes the purification and some properties of the adenosine phosphorylase of *B. subtilis*.

Materials and Methods

Materials

Bovine serum albumin, egg albumin (type V), cytochrome *c* (horse heart, type III), and alkaline phosphatase (*Escherichia coli*) were obtained from Sigma Chemical Company (St. Louis, Mo., U.S.A.). Xanthine oxidase, glycogen phosphorylase *a* (rabbit muscle), catalase (horse heart), glyceraldehyde-3-phosphate dehydrogenase (rabbit muscle), alcohol dehydrogenase (yeast), and α-chymotrypsinogen were from Boehringer (Mannheim, Germany). Uridine phosphorylase (*E. coli*) and thymidine phosphorylase (*E. coli*) were gifts from K. Hammer-Jespersen [10] and M. Schwartz [11], respectively. Other chemicals (reagent grade) were purchased from Merck (Darmstadt, Germany). Ribose 1-phosphate was prepared from inosine [12].

Bacterial strain and growth procedure

B. subtilis QB-16 was grown in Antibiotic Medium 3 (Difco) at 37°C with vigorous aeration. When the culture was in the exponential phase (about $2 \cdot 10^8$ cells per ml), it was chilled by addition of ice. The cells were harvested in a Sharples centrifuge.

Enzyme assays

All enzyme assays were carried out at 37°C. One unit of enzyme catalyzes the formation of 1 μmol product/min at standard assay conditions.

Procedure A. Phosphorolysis of inosine was measured spectrophotometrically as described before [3]. The substrate concentrations in the standard assay of the inosine-guanosine phosphorylase were 0.18 M potassium phosphate (pH 7.1) and 2.0 mM inosine.

Procedure B. The colorimetric determination of the phosphorolysis of deoxynucleosides has been described previously [3]. In the standard assay of the adenosine phosphorylase the reactions contained 20 mM potassium phosphate (pH 7.1) and 1.0 mM deoxyadenosine. At 10 s, 2, 5 and 10 min 0.35 ml samples were withdrawn for determination of deoxyribose 1-phosphate [3].

Analytical polyacrylamide gel electrophoresis

Electrophoresis at pH 10.3 was performed using the discontinuous Tris/glycine system described by Davis [13]. The gels had the dimensions 0.6 × 8 cm and were run at 2.5 mA (constant current). In addition, electrophoresis was carried out at pH 6.5. These latter gels contained 7.5% acrylamide and were

made as described by Weber and Osborn [14] for dodecyl sulfate gels, but with dodecyl sulfate omitted. 0.6 × 8 cm gels were run at 120 V (constant voltage). Electrophoresis of dodecyl sulfate-denatured proteins was performed as described [14]. In all cases, the proteins were stained with Coomassie Brilliant Blue G-250.

Preparative acrylamide gel electrophoresis

This was performed in a "Poly-prep" apparatus from Buchler. The Tris/glycine system originally described by Ornstein and Davis [13] was used in the modification for preparative use described in the "Poly-prep" manual. The upper gel was omitted. The size of the gel was 100 ml. The electrophoresis was carried out at 50 mA (constant current) and at 5°C. The lower buffer was used as elution buffer (flow rate 63 ml/h; 6.3 ml fractions).

Results

Two purine nucleoside phosphorylases in B. subtilis.

Extracts of *B. subtilis* were subjected to analytical polyacrylamide gel electrophoresis. Regions of the gels containing purine nucleoside phosphorylase activities towards inosine and adenosine were visualized [15]. Two well-spaced activity bands were seen (Fig. 1); one specific for inosine and the other for adenosine. This confirms the observation of Senesi et al. [6] that *B. subtilis* contains two purine nucleoside phosphorylases of different specificities.

Purification

All operations were carried out at 0—4°C. The centrifugations were carried out at 25 000 × *g* for 30 min.

(1) Preparation of crude extracts. 28 g wet weight of *B. subtilis* cells were

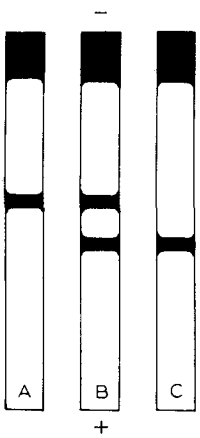

Fig. 1. Location of purine nucleoside phosphorylases in polyacrylamide gels of extracts of *B. subtilis*. To each of the gels (pH 10.3) was applied 30 μg of the DE-52 pool (Fraction 2) containing 8% sucrose. After electrophoresis at 5°C the gels were incubated for 1 h at 37°C with 0.1 M potassium arsenate pH 7.1 containing 1 mM adenosine (gel A), 1 mM inosine and 1 mM adenosine (gel B), or 1 mM inosine (gel C). After the incubation the gels were stained with 2,3,5-triphenyltetrazolium as described [15]. The staining at the top of the gels is due to the sucrose in the sample.

suspended in 67 ml 50 mM Tris · HCl (pH 7.2)/2 mM EDTA and disrupted by sonication for 5 × 1 min with a Branson sonifier. Debris was removed by centrifugation. To the supernatant was added 5 mM β-mercaptoethanol and 50 mM NaCl (Fraction 1).

(2) Chromatography on DE-52 cellulose. Fraction 1 was pumped onto a 2.5 × 40 cm column of DE-52 cellulose equilibrated with 50 mM Tris · HCl (pH 7.5)/2 mM EDTA/5 mM β-mercaptoethanol/50 mM NaCl (flow rate 63 ml/h). The column was washed with 100 ml of the same buffer. The purine nucleoside phosphorylases were eluted with a gradient from 50 to 450 mM NaCl in the buffer described above. The total volume of the gradient was 2 l; 10.5 ml fractions were collected. Fractions 60—83 containing the two phosphorylases were pooled and dialysed against 35 ml potassium phosphate (pH 6.8)/1 mM β-mercaptoethanol/0.1 mM EDTA. The volume then was 254 ml (Fraction 2).

(3) Chromatography on hydroxyapatite. Fraction 2 was applied to a 2.5 × 18 cm column of hydroxyapatite equilibrated with 35 mM potassium phosphate (pH 6.8)/1 mM β-mercaptoethanol/0.1 mM EDTA (flow rate 33 ml/h). The column was washed with 110 ml of the same buffer and then eluted with a 35—250 mM gradient in potassium phosphate (total volume 1 l). 11-ml fractions were collected from the start of the gradient. The adenosine phosphorylase peaked in Fraction 22 (52 mM potassium phosphate) and the inosine-guanosine phosphorylase peaked in fraction 30 (73 mM potassium phosphate). Fractions 20—38 were pooled (183 ml) and precipitated with 123 g solid $(NH_4)_2SO_4$. The precipitate was dissolved in 10 mM Tris · HCl (pH 7.5)/0.2 mM EDTA/2 mM β-mercaptoethanol and dialysed against the same buffer. The volume after dialysis was 20 ml (Fraction 3).

(4) Preparative electrophoresis. To Fraction 3 was added sucrose to a final concentration of 5% and Bromphenol Blue. This solution was applied to a "Poly-prep" apparatus and electrophoresis was started. The collection of fractions was begun just prior to the elution of Bromphenol Blue. This compound eluted in Fraction 7. The inosine-guanosine phosphorylase activity peaked in Fraction 31, and the adenosine phosphorylase peaked in Fraction 48. The two enzymes were separated by about 10 fractions at the foot of the peaks.

Steps to purify further the adenosine phosphorylase

Isoelectric focusing, pH 5—7 (A 5). Fractions 46—51 from the preparative electrophoresis were pooled (39 ml) and precipitated with 26 g solid $(NH_4)_2SO_4$. The precipitate was spun down and dissolved in 10 mM Tris · HCl (pH 7.8)/1 mM EDTA/1 mM β-mercaptoethanol and dialysed against the same buffer. The volume was 5.2 ml (Fraction A 4). Isoelectric focusing was carried out using an LKB 8100-1 Ampholinic Electrofocusing Column at 5°C according to the LKB manual. 110 ml 1.5% solution of Ampholines (pH range 5—7) was stabilized by a 0—75% gradient of ethylene glycol. The cathode (bottom) solution was 25 ml 0.12 M NaOH in 60% sucrose. The anode (top) solution was 10 ml 0.15 M phosphoric acid. The focusing was carried out at 960 V for 48 h. The gradient was tapped in 3-ml fractions from the bottom. Adenosine phosphorylase peaked on the very steep pH gradient close to the anode solution (about pH 4.6). 6 fractions were pooled and dialysed against the buffer described above. Proteins in the dialysed fraction (28 ml) were precipitated

with 17.6 g solid $(NH_4)_2SO_4$, dissolved in 2 ml of the buffer and dialysed. The volume was 2.5 ml (Fraction A 5).

Chromatography on DEAE-Sephadex (A 6). Fraction A 5 was applied to a 0.9 × 6 cm column of DEAE-Sephadex equilibrated with 25 mM Tris/25 mM succinate (pH 6.9)/0.5 mM EDTA/1 mM β-mercaptoethanol. The column was washed with 10 ml of the same buffer. Adenosine phosphorylase was eluted with a gradient (total volume 200 ml) from 0 to 1 M NaCl. (flow rate 8.2 ml/h; 2.3-ml fractions). The enzyme appeared at about 0.4 M NaCl. 5 fractions containing most of the activity were pooled and precipitated with 7.1 g solid $(NH_4)_2SO_4$. The pellet was dissolved in 1 ml 10 mM Tris · HCl (pH 7.8)/1 mM β-mercaptoethanol/1 mM EDTA and dialysed against the same buffer (Fraction A 6).

Table I summarizes the purification of the adenosine phosphorylase.

Steps to further purify the inosine-guanosine phosphorylase

Fractions 30—33 from the preparative electrophoresis were pooled (26 ml) and precipitated with 18.2 g solid $(NH_4)_2SO_4$ (95% saturation). The pellet was dissolved in and dialysed against 10 mM Tris · HCl (pH 7.8)/1 mM β-mercaptoethanol/1 mM EDTA. The volume after dialysis was 5.1 ml (Fraction IG 4). To this solution was added 1.9 g solid $(NH_4)_2SO_4$ (60% saturation). The precipitate was spun down and the supernatant was dialysed against 25 mM Tris/25 mM succinate (pH 6.9)/1 mM β-mercaptoethanol/0.5 mM EDTA and chromatographed on a DEAE-Sephadex column as described for adenosine phosphorylase. The activity eluted at about 0.3 M NaCl. 3 peak fractions were pooled (6.6 ml) and precipitated with 4.5 g $(NH_4)_2SO_4$. The pellet was dissolved in and dialysed against 10 mM Tris · HCl (pH 7.8)/1 mM EDTA/2 mM β-mercaptoethanol (Fraction IG 5, 1.2 ml).

Table II summarizes the purification of the inosine-guanosine phosphorylase.

Purity of the enzymes

The adenosine phosphorylase (Fraction A 6) appeared homogeneous by electrophoresis at pH 6.5 (Fig. 2). By electrophoresis at pH 10.3 a weak protein band was seen besides the main band, which corresponded to the adenosine

TABLE I

PURIFICATION OF THE ADENOSINE PHOSPHORYLASE FROM *B. SUBTILIS*

The amount of protein in the first three fractions was determined by the method of Lowry et al. [16]. For fractions A4, A5, and A6 one absorbance unit at 280 nm was considered equivalent to a protein concentration of 1 mg/ml.

Fraction	Treatment	Protein	Specific activity (units/mg)	Purification (-fold)	Recovery (%)
1	Crude extract	2470	0.09	1.0	100
2	DE-52 cellulose	790	0.20	2.3	74
3	Hydroxyapatite	135	0.99	11	61
A4	Preparative electrophoresis	7	13	145	41
A5	Isoelectric focusing	1.1	33	373	17
A6	DEAE-Sephadex	0.2	73	830	8

TABLE II

PURIFICATION OF THE INOSINE-GUANOSINE PHOSPHORYLASE FROM *B. SUBTILIS*

The amount of protein in the first three fractions was determined by the method of Lowry et al. [16]. For fractions A4, A5, and A6 one absorbance unit at 280 nm was considered equivalent to a protein concentration of 1 mg/ml.

Fraction	Treatment	Protein	Specific activity (units/mg)	Purification (-fold)	Recovery (%)
1	Crude extract	2470	0.15	1.0	100
2	DE-52 cellulose	790	0.26	2.5	78
3	Hydroxyapatite	135	1.67	11	63
IG4	Preparative electrophoresis	2.6	50	340	36
IG5	60—95% ammonium sulfate, DEAE-Sephadex	0.6	100	690	17

phosphorolytic activity (Fig. 2). In dodecyl sulfate gels, one strong and three weak protein bands were seen (Fig. 2). Thus, this enzyme was 70—80% pure.

The inosine-guanosine phosphorylase (Fraction IG 5) was about 40% pure. By electrophoresis at pH 6.5 two about equally strong protein bands (R_F = 0.35 and 0.43) and a few weak bands were seen. The inosine phosphorylase activity coincided with the faster migrating of the two major bands. In dodecyl sulfate gels also two major and some very weak bands were seen (unpublished data).

Properties of the enzymes

Specificities. After purification of the enzymes (using fractions A 4 and IG 4), their specificity was tested. The results are presented in Table III. Adenosine phosphorylase was virtually specific for adenosine and deoxyadenosine, while the inosine-guanosine phosphorylase used hypoxanthine and guanine

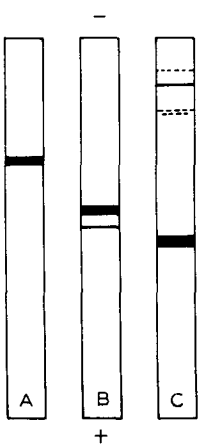

Fig. 2. Analytical polyacrylamide gel electrophoresis of adenosine phosphorylase fraction A 6. (A), gel (pH 6.5) containing native protein (10 μg). (B), gel (pH 10.3) containing native protein (10 μg). (C), gel containing dodecyl sulfate denatured protein (10 μg).

TABLE III

SPECIFICITIES OF THE PURINE NUCLEOSIDE PHOSPHORYLASE FROM *B. SUBTILIS*

The reactions contained in a total of 1.00 ml: 20 mM potassium arsenate pH 7.1, 1.0 mM nucleoside, and one of the purine nucleoside phosphorylases. They were incubated at 37°C; at 10 s, 5 min, 10 min, 30 min 100-μl aliquots were withdrawn for determination of reducing sugar. For the inosine-guanosine phosphorylase the rate of aresenolysis of guanosine (12 nmol/min per ml) is set to be 100%. For the adenosine phosphorylase the rate of aresenolysis of deoxyadenosine (21 nmol/min per ml) is set to be 100%.

Nucleoside	% velocity	
	Inosine-guanosine phosphorylase	Adenosine phosphorylase
Inosine	83	1
Adenosine	<1	24
Guanosine	100	1
Xanthosine	1	<1
Uridine	<1	<1
Deoxyinosine	63	<1
Deoxyadenosine	<1	100
Deoxyguanosine	93	4
Deoxyuridine	<1	<1

nucleosides, but not adenine and xanthine nucleosides as substrates. The specificities were confirmed by the experiment shown in Fig. 3. For the inosine-guanosine phosphorylase (Fig. 3A) with deoxyinosine as substrate, inosine and guanosine were strong competitive inhibitors, while adenosine had little or no

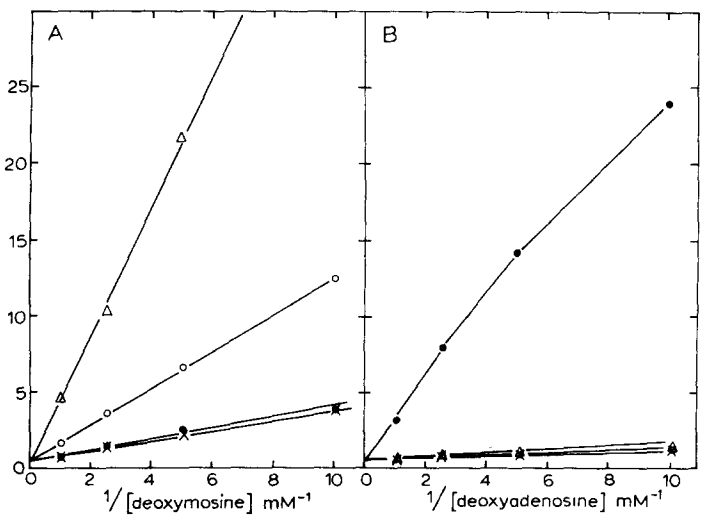

Fig. 3. Inhibition of phosphorolysis of deoxynucleosides by ribonucleosides. The reactions contained in 2.0 ml total volume: 20 mM potassium phosphate, pH 7.1, deoxynucleoside as indicated, and 0.03 units of inosine-guanosine phosphorylase (A) or 0.015 units of adenosine phosphorylase (B). In addition, the reactions contained: no further addition (X), 0.5 mM adenosine (•), 0.5 mM inosine (○), or 0.5 mM guanosine (△). At various times 0.35 ml samples were withdrawn for determination of deoxyribose 1-phosphate (Assay Procedure B). The velocity is expressed as nmol/min/0.35 ml and the reciprocal is here depicted vs. the reciprocal of the deoxynucleoside concentration. For the inosine-guanosine phosphorylase the kinetic constants are: K_m (deoxyinosine) = 0.9 mM; K_i (inosine) = 0.20 mM; K_i (guanosine) = 0.05 mM; and K_i (adenosine) = 4 mM. For the adenosine phosphorylase the kinetic constants are: K_m (deoxyadenosine) = 0.09 mM; K_i (adenosine) = 0.05 mM; K_i (inosine) $\geqslant$ 2 mM; and K_i (guanosine) $\geqslant$ 0.9 mM.

effect. For adenosine phosphorylase (Fig. 3B), with deoxyadenosine as substrate, adenosine was a potent competitive inhibitor, while only slight inhibition was observed with inosine and guanosine.

The inosine-guanosine phosphorylase

Further characterization of the inosine-guanosine phosphorylase revealed apparent K_m values of 0.2 and 3.9 mM for inosine and phosphate, respectively. The gel filtration behaviour of the native enzyme was consistent with a mol. wt. of 95 000. After elution from an analytical acrylamide gel (pH 6.5) of the protein band corresponding to the inosine phosphorolytic activity, a subsequent dodecyl sulfate gel showed a mol. wt. of 28 000 for the subunit. These results indicate that the inosine-guanosine phosphorylase of *B. subtilis* is very similar to the homologous enzyme of *B. cereus* [7—9], which has been shown to be a tetramer of mol. wt. 95 000 [9].

The adenosine phosphorylase

Lineweaver-Burk plots. Fig. 3B indicates that the adenosine phosphorylase did not obey Michaelis-Menten kinetics with respect to deoxyadenosine. As shown in Fig. 4, the Lineweaver-Burk plots for both phosphate and deoxyadenosine are non-linear. A Hill plot of the data for deoxyadenosine (unpublished data) was linear in the range 0.02—0.5 mM with a Hill coefficient (n_H) of 0.78 and a Hill constant of 0.09 mM.

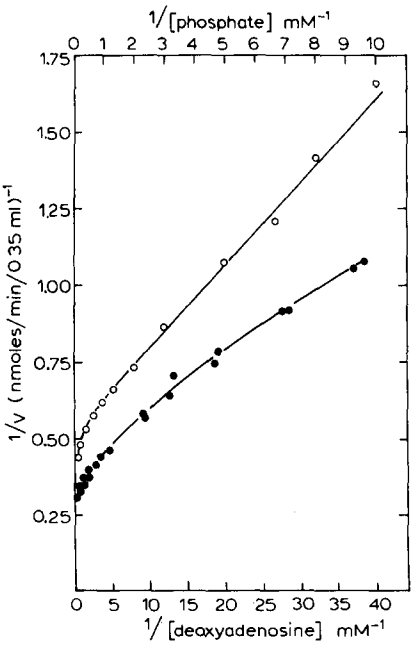

Fig. 4. Lineweaver-Burk plots of the adenosine phosphorylase activity with phosphate or deoxyadenosine as variable substrates. With deoxyadenosine (●———●) as the variable substrate the reactions (2.0 ml) contained: 20 mM potassium phosphate pH 7.1, 17 mU of adenosine phosphorylase, and deoxyadenosine as indicated. With phosphate as the variable substrate (○———○) the reactions (2.0 ml) contained: 50 mM Tris · HCl pH 7.1, 14 mU adenosine phosphorylase, and potassium phosphate as indicated. The deoxyribose 1-phosphate formed was determined as described under Assay Procedure B.

Effects of p-chloromercuribenzoate. *p*-Chloromercuribenzoate inactivated adenosine phosphorylase; this inactivation could be completely reversed by β-mercaptoethanol (Table IV). After 10 min at 37°C in the presence of 10^{-3} M *p*-chloromercuribenzoate, a significant portion (13%) of the activity remained. Phosphate, adenosine, and ribose 1-phosphate protected the enzyme against *p*-chloromercuribenzoate, while adenine had no effect.

Molecular weight and subunit structure. Adenosine phosphorylase sedimented at 0—2°C in 5—20% sucrose gradients (3 gradients) for 27 h at 38 000 rev./min using a Beckman ultracentrifuge (L2-65B) equipped with an SW 41 rotor. Yeast alcohol dehydrogenase ($s_{20,w}$ = 7.61 [17]) and uridine phosphorylase ($s_{20,w}$ = 8.2 [10]) were included as markers. From these experiments (unpublished data) an $s_{20,w}$ of 7.80 ± 0.06 was determined for adenosine phosphorylase. According to Martin and Ames [18] this should correspond to a molecular weight of 150 000—160 000 for the enzyme.

The molecular weight of the native enzyme was also estimated by gel filtration on a Sephadex G-200 column using pure proteins as markers [19]. Adenosine phosphorylase eluted as a protein of mol. wt. 153 000 ± 10%. In dodecyl sulfate gels [14] adenosine phosphorylase migrated as a polypeptide with the mol. wt. 25 500 ± 5%. This assumes that the predominant band (Fig. 2, gel C) represents this enzyme. This assumption is quite plausible since purine nucleoside phosphorylases usually have subunits about this size [2,3]. The three weak bands in the dodecyl sulfate gels correspond to mol. wt. > 65 000 and subunits of this large size have never been reported for any purine nucleoside phosphorylase. Thus, these data indicate a hexameric structure for adenosine phosphorylase of *B. subtilis*.

TABLE IV

EFFECT OF *p*-CHLOROMERCURIBENZOATE (*p*Cl-HgBzOH) ON ADENOSINE PHOSPHORYLASE WITH VARIOUS SUBSTRATES PRESENT

0.10-ml aliquots were mixed on ice. They contained 0.10 M Tris · HCl pH 7.8, 0.1 mM EDTA, substrates as indicated, and 0.01 units of adenosine phosphorylase. After incubation at 37°C for 5 min 10 μl of a 10 mM *p*Cl-HgBzOH in 15 mM KOH (B) or 10 μl 15 mM KOH (A) were added. After another 10 min at 37°C 1.90 ml of a solution of deoxyadenosine (0.25 mM) in potassium phosphate (20 mM) pH 7.1 were added and the reaction velocities were determined by assay procedure B (see Materials and Methods). 100% activities were determined in each case due to some inhibition from the added substrates on the subsequent phosphorolysis of deoxyadenosine.

Substrate(s) added	Velocities (nmol/min per 0.35 ml)		% activity $B/A \times 100\%$
	—*p*Cl-HgBzOH (*A*)	$+10^{-3}$ M *p*Cl-HgBzOH (*B*)	
No substrates	0.94	0.12	13%
1.0 mM adenosine	0.47	0.30	64%
10 mM phosphate	1.02	0.72	71%
1.0 mM ribose 1-phosphate	0.83	0.67	81%
1.0 mM adenine	0.64	0.08	13%
1.0 mM adenosine + 10 mM phosphate	(0.47)	0.37	79%
No substrates *	0.94	0.91	97%

* In this incubation 14 mM 2-mercaptoethanol was added after incubation for 10 min with *p*Cl-HgBzOH and the reactions were left for another 10 min before determination of activity.

Discussion

The results presented confirm the observation of Senesi et al. [6] that vegetative cells of *B. subtilis* contain two purine nucleoside phosphorylases: one specific for adenine nucleosides and the other specific for guanine and hypoxanthine nucleosides.

The adenosine phosphorylase from *B. subtilis* elutes from Sephadex G-200 columns as a protein with the molecular weight 153 000 ± 10%. The molecular weight of its subunit appears to be 25 500 ± 5% suggesting a hexameric structure. The adenosine phosphorylase is relatively resistant to *p*-chloromercuribenzoate. At 10^{-3} M *p*-chloromercuribenzoate the enzyme is slowly inactivated. Adenosine, phosphate, and ribose 1-phosphate, but not adenine protect against this inactivation. These data suggest a reaction mechanism where phosphate, nucleoside, and pentose 1-phosphate are all able to bind to the free enzyme. Such a mechanism has been proposed for the purine nucleoside phosphorylase of enteric bacteria [20].

Nucleoside hydrolases able to split adenosine hydrolytically have been observed in organisms [1] including *B. cereus* [21]. These enzymes are frequently stimulated by phosphate [1]. The phosphorolytic nature of the adenosine-splitting activity described herein and by Senesi et al. is proven by the fact that the enzyme catalyzes the formation of adenosine from adenine and ribose 1-phosphate [14], (not shown).

It is interesting to note that the inosine-guanosine phosphorylase of *B. subtilis* is a tetramer, while the adenosine phosphorylase is a hexamer. Apart from being specific for adenine nucleosides this latter enzyme is very similar to the general purine nucleoside phosphorylase from *Salmonella typhimurium* and *E. coli* [3,4,20]. The similarities include: the ability to cleave adenosine at a high rate; the molecular weight of the native enzymes and their subunits; the requirement for a rather high concentration (10^{-3} M) of *p*-chloromercuribenzoate in order to inactivate the enzymes; and the pattern of protection by substrates against the action of *p*-chloromercuribenzoate, notably the protection (binding) by (of) ribose 1-phosphate.

No enzyme similar to the *B. subtilis* adenosine phosphorylase has been found in other organisms. However, it seems not unlikely that a similar enzyme could be present in *B. cereus*. In all cases where purine nucleoside phosphorylase from *B. cereus* was studied [7–9] a preparative gel electrophoresis was incorporated in the purification procedure. The enzyme was purified according to inosine phosphorolysis and only after the purification was the specificity tested. The preparative electrophoresis may well have removed any trace of adenosine phosphorolytic activity. For human cells indirect evidence suggests that an adenosine splitting activity might occur [2], but the enzyme responsible has not been found.

Acknowledgments

The author wishes to thank Dr. J. Neuhard for suggesting this investigation and for bacteriological advice, and Dr. P. Nygaard for reading the manuscript.

References

1 Friedkin, M. and Kalckar, H. (1961) in The Enzymes, 2nd edn. (Boyer, P.D., Lardy, H. and Myrbäck, K., eds.), Vol. 5, pp. 237—255, Academic Press, New York
2 Parks, Jr., R.E. and Agarwal, R.P. (1972) in The Enzymes, 3rd edn. (Boyer, P.D., ed.), Vol. 7, pp. 483—514, Academic Press, New York
3 Jensen, K.F. and Nygaard, P. (1975) Eur. J. Biochem. 51, 253—265
4 Robertson, B.C. and Hoffee, P.A. (1973) J. Biol. Chem. 248, 2040—2043
5 Hatanaka, M., Del Giudice, R. and Long, C. (1975) Proc. Natl Acad. Sci. U.S. 72, 1401—1405
6 Senesi, S., Falcone, G., Mura, U., Sgarrella, F. and Ipata, P.L. (1976) FEBS. Lett. 64, 353—357
7 Gardner, R. and Kornberg, A. (1967) J. Biol. Chem. 242, 2383—2388
8 Engelbrect, K.L. and Sadoff, H.L. (1969) J. Biol. Chem. 244, 6228—6232
9 Gilpin, R.W. and Sadoff, H.L. (1971) J. Biol. Chem. 246, 1475—1480
10 Leer, J.C., Hammer-Jespersen, K. and Schwartz, M. (1977) Eur. J. Biochem. 75, 217—224
11 Schwartz, M. (1971) Eur. J. Biochem. 21, 191—198
12 Jensen, K.F., Leer, J.C. and Nygaard, P. (1973) Eur. J. Biochem. 40, 345—354
13 Davis, B.J. (1964) Ann. N.Y. Acad. Sci. 121, 404—427
14 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406—4412
15 Gabriel, O. and Wang, S.F. (1969) Anal. Biochem. 27, 545—554
16 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 256—265
17 Bühner, M. and Sund, H. (1969) Eur. J. Biochem. 11, 73—79
18 Martin, R.G. and Ames, B.N. (1961) J. Biol. Chem. 236, 1372—1379
19 Andrews, P. (1965) Biochem. J. 96, 595—606
20 Jensen, K.F. (1976) Eur. J. Biochem. 61, 377—386
21 Powell, J.F. and Hunter, J. R. (1956) Biochem. J. 62, 381—387
22 Snyder, F.F. and Henderson, J.F. (1973) J. Biol. Chem. 248, 5899—5904