Biochimica et Biophysica Acta, 293 (1973) 359–369

BBA 66822

IMMUNOCHEMICAL STUDIES OF AMP:PYROPHOSPHATE PHOSPHORIBOSYLTRANSFERASE FROM NORMAL AND LESCH–NYHAN SUBJECTS

LILY C. YIP, JOSEPH DANCIS AND M. EARL BALIS

Division of Cell Metabolism, Sloan-Kettering Institute for Cancer Research, New York, N.Y. 10021 and Department of Pediatrics, New York University School of Medicine, New York, N.Y. 10016 (U.S.A.)

(Received August 28th, 1972)

SUMMARY

Adenine phosphoribosyltransferase (AMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.7) has been purified 4000-fold from human erythrocytes. On Sephadex column chromatography or polyacrylamide gel electrophoresis multiple peaks were observed. The prior addition of 5-phosphoribosyl-1-pyrophosphate (*P*-Rib-*P*-*P*) to the enzyme produced a single peak with a molecular weight of 26 000. Magnesium is not required to form the *P*-Rib-*P*-*P* adenine phosphoribosyltransferase complex. The complex is more stable to heat denaturation.

Rabbit antiserum against human adenine phosphoribosyltransferase cross-reacts with lysate of erythrocytes of rhesus monkey but not with those of rabbit, dog, mouse and sheep. Neutralization of rhesus adenine phosphoribosyltransferase is more effective than of human enzyme indicating structural differences between the enzymes.

The antiserum neutralizes the same amount of adenine phosphoribosyltransferase activity in erythrocytes from Lesch–Nyhan patients and normal subjects. The enzymes from both sources are not distinguishable by radial diffusion against antiserum or by immunoelectrophoresis. This evidence of immunological identity is consistent with the postulate that the elevated levels of adenine phosphoribosyltransferase found in Lesch–Nyhan patients are due to stabilization of the enzyme by endogenous metabolites.

INTRODUCTION

The Lesch–Nyhan syndrome is a neurological disorder in which the afflicted individuals have an aberrant purine metabolism[1,2]. The primary metabolic defect is an absence of hypoxanthine phosphoribosyltransferase (IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8). The defect is evident in the erythrocyte where it is

Abbreviation: *P*-Rib-*P*-*P*, 5-phosphoribosyl-1-pyrophosphate.

associated with an abnormally high level of activity of adenine phosphoribosyltransferase (AMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.7)[3]. Fractionation of erythrocytes according to age has permitted the demonstration that the elevated adenine phosphoribosyltransferase results from a prolonged half-life rather than increased synthesis[4]. The increased *in vivo* stability of the enzyme is associated with an increased heat stability *in vitro*.

The high adenine phosphoribosyltransferase activity in erythrocytes of Lesch–Nyhan patients could be the result of a pleiotropic effect of the mutation causing preferential synthesis of stable isozymes, or of stabilization of the enzyme by endogenous effectors. Earlier studies with partially purified adenine phosphoribosyltransferase favored the latter interpretation[4]. However, multiple forms of adenine phosphoribosyltransferase have been demonstrated in intestinal epithelial cells[5].

The identification of multiple enzyme forms has been approached immunochemically, by many authors[6–8]. In the present study, we have purified adenine phosphoribosyltransferase from human erythrocytes and prepared antibodies in rabbits. The antisera have been used to study the enzyme in normal humans and in patients with Lesch–Nyhan disease. Adenine phosphoribosyltransferase from other animal species have also been examined.

METHODS

Enzyme assay

A modification of previously described methods was used[9,10]. A solution containing 10 μmoles of Tris, pH 8.0, 0.05 μmoles of *P*-Rib-*P*-*P* as sodium salt and 1 μmole of Mg^{2+} was mixed with 50 μl of an enzyme solution. The reaction was started by adding 50 μl of 1 mM [8-^{14}C]adenine (spec. act., 3.3 Ci/mole). The reaction mixture was incubated at 37 °C for an appropriate length of time, and the incubation terminated by immersion in ethanol–solid CO_2 bath. Nucleotide formation was measured as follows: 25 μl of the reaction mixture was spotted on DEAE-cellulose paper 2 cm^2 (Whatman DE81). Radioactive precursor was eluted by repeatedly washing with 0.002 M ammonium formate solution, water and absolute ethanol, in sequence. The paper was dried, immersed in 15 ml of scintillation fluid (0.01% 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene, 0.5% 2,5-diphenyloxazole in toluene) and radioactivity determined.

Protein assay

Hemoglobin concentration was determined as methemoglobin cyanide as described by Van Kampen and Zylstra[11]. Total protein was determined by the method of Lowry *et al.*[12].

Enzyme purification

All the following procedures were carried out at 4 °C, and were adapted from the method of partial purification previously reported[13].

Removal of hemoglobin

Hemoglobin was removed by passing lysate of out-dated erythrocytes (N.Y. Blood Center) through DEAE-cellulose[14].

Salt fractionation

Adenine phosphoribosyltransferase was precipitated from 45–70% saturated $(NH_4)_2SO_4$ solution. The precipitate was dissolved in a minimal volume of 0.01 M Tris–HCl (pH 7.4)–0.1 M KCl–0.001 M 2-mercaptoethanol–1.5% glycerol buffer.

Sephadex G-150 chromatography

The enzyme solution was applied to a 5.0 cm × 87 cm Sephadex G-150 column equilibrated with 0.01 M Tris–HCl (pH 7.4)–0.1 M KCl–0.001 M mercaptoethanol–1.5% glycerol. Void volume of the column was 570 ml. Ascending gel filtration was performed at a rate of 15 ml/h, and 10-ml fractions were collected. Fractions with maximum specific activity were pooled and dialyzed against 0.01 M potassium phosphate (pH 6.4)–0.02 M KCl–0.001 M mercaptoethanol–1.5% glycerol.

DEAE-cellulose column

The dialyzed enzyme preparation was applied to a DEAE-cellulose column (2 cm × 20 cm) equilibrated with the dialysis buffer. Enzyme was eluted with a linear gradient of KCl from 0.02 to 0.2 M at a flow rate of 30 ml/h. 5-ml fractions were collected. Highly active fractions were pooled and concentrated by 70% saturated $(NH_4)_2SO_4$ precipitation. The precipitate was then redissolved in a minimal amount of Tris–HCl (pH 7.4)–0.1 M KCl–0.001 M mercaptoethanol–1.5% glycerol.

Sephadex G-100 chromatography

The concentrated enzyme solution was applied to a Sephadex G-100 column (2.5 cm × 90 cm) equilibrated with the same Tris–HCl buffer. Ascending gel filtration was performed at a flow rate of 10 ml/h, and 3.5-ml fractions were collected. The active pool of the enzymes was once again concentrated by 70% saturated $(NH_4)_2SO_4$. The precipitate was redissolved and dialyzed against 0.01 M potassium phosphate (pH 7.0)–0.02 M KCl–0.001 M mercaptoethanol–1.5% glycerol.

DEAE Sephadex column fractionation

The dialyzed enzyme preparation was put on a DEAE-Sephadex A-50 column (1.2 cm × 20 cm) previously equilibrated with the same dialysis buffer. Enzyme was eluted with a linear gradient of KCl from 0.02 to 0.2 M at a flow rate of 5 ml/h. 3-ml fractions were collected. Highly active fractions were pooled and stored in 40% sucrose solution. Rabbit antiserum against human adenine phosphoribosyltransferase was prepared with this purified enzyme preparation.

Sephadex G-25 chromatography for heat stability study

A Sephadex G-25 column (1 cm × 12 cm) equilibrated with phosphate buffered saline was used. Void volume of the column was 2.3 ml. The flow rate was 0.2 ml/min. Sample size was 0.5 ml. Enzyme activity appeared within the 2 ml following the void volume.

Estimation of enzyme molecular weight

A 0.01 M Tris–HCl (pH 7.4)–0.1 M KCl–0.001 M mercaptoethanol–1.5% glycerol equilibrated Sephadex G-150 column (2.5 cm × 35 cm) was used. The column had a void volume of 65.4 ml, and was previously calibrated with molecular weight

markers. Ascending gel filtration was performed at a flow rate of 5 ml/h, 1.2-ml fractions. The molecular weight of the applied sample was estimated using a plot of K_{av} against the molecular weights of some known proteins, where

$$K_{av} = \frac{V_e - V_0}{V_t - V_0}$$

and V_e, elution volume for the protein; V_0, elution volume for Blue Dextran 2000; V_t, total bed volume.

Preparation of antisera

2 ml of the purified enzyme (100 μg) were mixed with an equal volume of Freund's adjuvant. The emulsion was injected subcutaneously into four sites on a rabbit's back. The injections were repeated four times at 7-day intervals and serum collected on the 42nd day after the first injection.

Immunodiffusion

About 2 μl of antiserum were placed in the center well of an Ouchterlony agar plate (Hyland) and the test antigens placed in the surrounding wells. After 24-h diffusion in a moist box at room temperature, the plates were washed with phosphate buffered saline for a period of 48 h, and the result recorded.

Single radial immunodiffusion

A modification of the method of Mancini was used[15]. A 1% agar with 0.05 M NaCl, 0.005% NaN_3 and 0.015 M phosphate pH 8.2–8.3 containing six times diluted antiserum was prepared. 2 μl of blood lysate (of a serial dilution) of normal and Lesch–Nyhan patients were put into wells about 1 cm apart. The antigens were permitted to diffuse for 24 h in a moist chamber.

Polyacrylamide gel electrophoresis

Electrophoresis of 10- to 100-μl samples containing 50–400 μg of protein was performed by the modified method of Davis[16] with the omission of the sample polymerization step. Samples were layered above the stacking gel in 40% sucrose and the experiment was performed at 4 °C with 3 mA per tube at 200 V. After electrophoresis the gel was cut in half lengthwise. One half was stained for protein. The corresponding protein area on the other half of the gel was eluted with phosphate buffer 0.1 M, pH 7.4, and assayed for enzyme activity.

Immunoelectrophoresis

1% agar in EDTA, boric acid and Tris buffer, pH 8.2, was uniformly layered on a microscopic slide. After hardening, a well (diameter 2 mm) was punched out in the center of the agar plate and 2 μl of the enzyme or diluted blood lysate were placed in the well. Electrophoresis was performed on two identical slides (A and B) at room temperature with 2 mA per plate at 100 V for 2–3 h. After electrophoresis, troughs were cut on Slide A and antiserum layered into the troughs. Precipitation lines were read after 24 h diffusion in a moist chamber at room temperature. During this period Slide B was kept frozen. Then, the areas corresponding to the positions of the migrated

protein after electrophoresis, as estimated from the precipitation lines of Slide A, were cut, eluted and assayed.

Enzyme neutralization

An appropriate amount of rabbit anti-adenine phosphoribosyltransferase serum was mixed with different volumes of lysed cells or an equivalent amount of purified enzyme. In control mixtures bovine serum albumin solution replaced anti-serum. Phosphate buffered saline was added to a total volume of 200 μl. The mixture was incubated at 4 °C for 16 h and centrifuged at 2000 $\times$ *g* for 30 min. 50 μl of the supernatant were used for assay of adenine phosphoribosyltransferase activity.

Selection of Lesch–Nyhan patients

The typical clinical picture was present in the patients tested. The biochemical evidence in all patients included hyperuricosuria[1], inverted hypoxanthine/xanthine ratio[17], absence of erythrocyte hypoxanthine phosphoribosyltransferase activity, and an elevated adenine phosphoribosyltransferase activity in erythrocytes[3].

RESULTS

A 4000-fold purified adenine phosphoribosyltransferase has been obtained from human erythrocytes (Table I). The purified adenine phosphoribosyltransferase

TABLE I

PURIFICATION OF ADENINE PHOSPHORIBOSYLTRANSFERASE

	Total protein	*Protein mg/ml*	*Activity I.U./ml*	*Spec. act. I.U./mg*	*Purification factor*	*% Recovery of activity*
Hemolysate	76.6 g	191	0.0448	0.000235	—	—
DEAE batch	4.02	4.68	0.0192	0.004100	17.5	92
$(NH_4)_2SO_4$ (45–70%)	1.43	44.60	0.414	0.009250	39.6	69
Sephadex G-150	66.2 mg	0.413	0.0364	0.088000	374.4	32.4
DEAE column	24.3	0.450	0.0808	0.18000	765.0	24.4
Sephadex G-100	2.71	0.053	0.0216	0.40700	1730	6.2
DEAE Sephadex	1.02	0.051	0.0482	0.94000	4000	5.3

migrated as a single band on gel electrophoresis. Upon storage in 40% sucrose at 0 °C for a week, two additional slower moving bands appeared on electrophoresis, and 40% of the original enzyme activity was lost. All three bands contained enzyme activity. The stored purified enzyme, when applied to a calibrated Sephadex G-150 column, resolved into several active components (Fig. 1) with apparent molecular weights ranging from 18 000 to 45 000. However, if this same enzyme preparation was pre-incubated with *P*-Rib-*P*-*P* before it was applied to the Sephadex column, most of the enzyme activity appeared in the peak consistent with a molecular weight of 26 000. Protein concentration was too low to be measured by absorbance at 280 nm.

Heat stability of adenine phosphoribosyltransferase was determined at 50 °C (Table II). The prior addition of *P*-Rib-*P*-*P*, 10 mM, protected the enzyme against

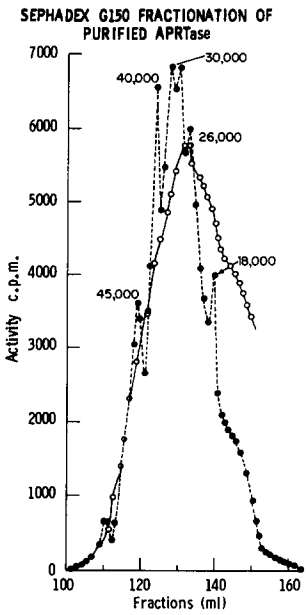

Fig. 1. Sephadex G-150 chromatography of purified adenine phosphoribosyltransferase (APRTase) with and without added *P*-Rib-*P*-*P*. 50 μg of the purified adenine phosphoribosyltransferase with and without added *P*-Rib-*P*-*P* (5 mg/ml) were passed through a calibrated Sephadex G-150 column. The addition of *P*-Rib-*P*-*P* converts the enzyme preparation from a mixture of apparently different molecular sizes to a single species with a molecular weight of approximately 26 000. ○—○, with *P*-Rib-*P*-*P*; ●---●, without *P*-Rib-*P*-*P*.

heat denaturation and the protection was retained after filtration through a Sephadex G-25 column. It appears that *P*-Rib-*P*-*P* is tightly bound to adenine phosphoribosyltransferase and the binding does not require the addition of Mg^{2+}, although the assay does.

Immunodiffusion of crude blood lysate from normal individuals against adenine phosphoribosyltransferase antiserum revealed a single precipitation line (Fig. 2A). With immunoelectrophoresis a weak second precipitation line appeared (Fig. 2B). Enzyme activity was obtained only from the eluate of the area corresponding to the major precipitation line. The minor line could represent a trace of cross-reactive contaminant or a small amount of inactivated enzyme. Immunodiffusion and immunoelectrophoresis of lysates of Lesch–Nyhan erythrocytes yielded similar results.

The adenine phosphoribosyltransferase in normal and Lesch–Nyhan erythrocytes was compared immunologically using neutralization techniques. The adenine phosphoribosyltransferase antiserum produced a typical parabolic neutralization curve[18] with partially purified enzyme (Table III). Prior absorption of the antiserum with purified enzyme removed the precipitating capacity of the antiserum against both the normal and Lesch–Nyhan crude enzyme preparations.

Erythrocyte lysates from Rhesus monkey, rabbit, dog, rat, mouse and sheep, were examined by immunodiffusion. Cross reaction with antihuman adenine phosphoribosyltransferase occurred only with monkey lysate (Fig. 2A). Similar results were obtained with the neutralization experiment (Table IV). Although the blood lysate of

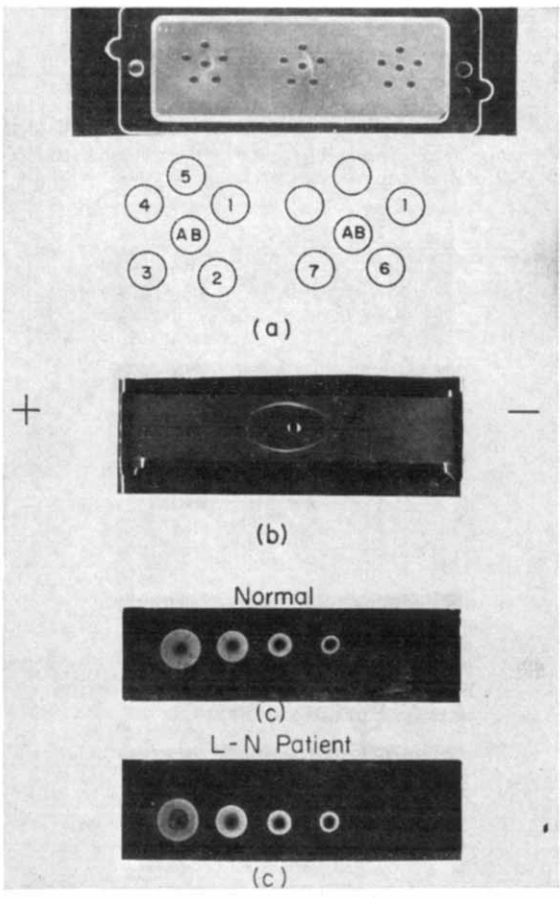

Fig. 2. (a) Immunodiffusion of blood lysates of different species with rabbit antiserum against human adenine phosphoribosyltransferase. Human (Well No. 1), Rhesus monkey (No. 2), rabbit (No. 3), rat (No. 4), mouse (No. 5), dog (No. 6) and sheep (No. 7). Rabbit anti-human adenine phosphoribosyltransferase serum was in the center well. A precipitate is seen only with human and monkey enzyme. (b) Immunoelectrophoresis of human blood lysate with rabbit antiserum against human adenine phosphoribosyltransferase. 2 μl of the normal blood lysate were applied as described in text. One major line is visible with a very faint second line. (c) Mancini's single radial immunodiffusion. Rabbit antiserum was diluted six times in the agar plate. The adenine phosphoribosyltransferase activities of the normal blood lysate applied in wells from left to right were $9.68 \cdot 10^{-3}$ I.U./ml, $7.74 \cdot 10^{-3}$ I.U./ml, $5.80 \cdot 10^{-3}$ I.U./ml, $3.87 \cdot 10^{-3}$ I.U./ml $1.93 \cdot 10^{-3}$ I.U./ml that of Lesch–Nyhan blood lysates were $10.2 \cdot 10^{-3}$ I.U./ml, $8.4 \cdot 10^{-3}$ I.U./ml, $6.2 \cdot 10^{-3}$ I.U./ml $4.2 \cdot 10^{-3}$ I.U./ml, and $2.08 \cdot 10^{-3}$ I.U./ml. There is no difference between normal and Lesch–Nyhan (L–N) lysate in the ratio of enzyme activity to antigenicity.

Rhesus monkey has higher adenine phosphoribosyltransferase activity than that of the human, as has been reported by Krenitsky[19], the antiserum neutralized the monkey adenine phosphoribosyltransferase more effectively (Table IV).

The specific activity of adenine phosphoribosyltransferase from normal and Lesch–Nyhan blood was determined by relating the amount of enzyme activity to the amount of enzyme protein as estimated by Mancini's single radial immunodiffusion method (Fig. 2C). There was no difference.

TABLE II

EFFECT OF *P*-Rib-*P*-*P* ON HEAT DENATURATION OF PARTIALLY PURIFIED ADENINE PHOSPHORIBOSYLTRANSFERASE

The enzyme purification was carried only through Step 1 and was approx. 17-fold purified. About 2 ml of enzyme solution were heated in a 50 °C water bath with constant shaking. Aliquots were removed at appropriate time intervals and immediately frozen in a solid CO_2–ethanol bath. Results show that a considerable amount of *P*-Rib-*P*-*P* is firmly bound.

Time at 50 °C (min)	*% Activity remaining*			
	Adenine phosphoribosyltransferase	*Adenine phosphoribosyltransferase + 10 mM P-Rib-P-P*	*Adenine phosphoribosyltransferase after Sephadex G-25 filtration*	*Adenine phosphoribosyltransferase + P-Rib-P-P after Sephadex G-25 filtration*
0	100	100	100	100
1	50	100	70	100
2	34	100	70	100
4	30	100	70	100
6	28	100	60	100
8	20	100	50	100
10	10	100	25	100

TABLE III

NEUTRALIZATION OF ADENINE PHOSPHORIBOSYLTRANSFERASE ACTIVITY OF NORMAL ERYTHROCYTE LYSATE BY RABBIT ANTI-ADENINE PHOSPHORIBOSYLTRANSFERASE SERUM

The 200 µl sample containing 20 µl of 1:10 lysate, varying amounts of antiserum and buffer was incubated at 4 °C for 16 h. After centrifugation at 2000 × *g* for 30 min, 50 µl of supernatant were assayed for adenine phosphoribosyltransferase activity. Results show parabolic neutralization curve.

Amount of antiserum (1:2 dil.) added (µl)	*Activity %*
—	100
5	100
10	45
20	9
40	21
80	63

When a fixed amount of rabbit anti-serum was mixed with serial dilutions of blood lysates from normal and Lesch–Nyhan patients, a parabolic inhibition pattern characteristic of a neutralization reaction was obtained (Fig. 3). Despite the greater specific activity and heat stability of adenine phosphoribosyltransferase from Lesch–Nyhan patients, no significant difference in immunological behavior of the blood lysates was found between normal and Lesch–Nyhan nor among different Lesch–Nyhan patients.

The immunological response of purified adenine phosphoribosyltransferase was the same in the presence or absence of *P*-Rib-*P*-*P* (Fig. 3). Thus, any change in conformation brought about by this stabilizer did not grossly change the antigenic site.

TABLE IV

NEUTRALIZATION OF ADENINE PHOSPHORIBOSYLTRANSFERASE ACTIVITY FROM DIFFERENT SPECIES BY ANTI-HUMAN ADENINE PHOSPHORIBOSYLTRANSFERASE SERUM

Heparinized blood samples were lysed with 10 vol. of 0.01 M potassium phosphate buffer. 10 μl of the blood lysates were incubated with equal volume of rabbit anti-human adenine phosphoribosyltransferase serum as described in Table III.

Species	*Inhibition (%)*
Human	70
*Rhesus monkey	92
Rabbit	<5
Rat	<5
Mouse	<5
Dog	<5
Sheep	<5

* Rhesus monkey blood lysate was further diluted (1:4) with buffer to obtain the same level of adenine phosphoribosyltransferase activity in control as that of human.

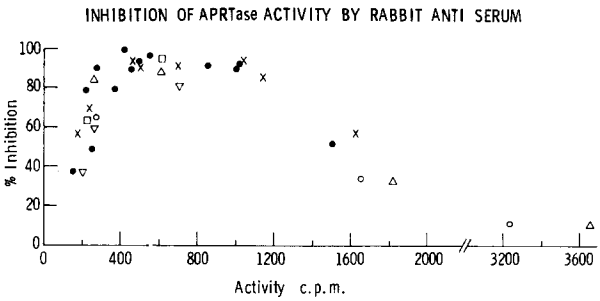

Fig. 3. Inhibition of adenine phosphoribosyltransferase (APRTase) by rabbit anti-adenine phosphoribosyltransferase serum. A fixed amount of anti-serum (10 μl) was used to neutralize a serially diluted blood lysate as described in the text. ●, Normal; ×, patient A, ○, Lesch–Nyhan patient S; △, Lesch–Nyhan patient W, purified enzyme in the presence of *P*-Rib-*P*-*P* (5 mg/ml); □, purified enzyme. There is no detectable difference in the inhibition curves.

DISCUSSION

Adenine phosphoribosyltransferase has been isolated and purified to various degrees from yeast[20], from Ehrlich ascites cells[21] and from extracts of *Bacillus subtilis*. The molecular weight of adenine phosphoribosyltransferase from *B. subtilis* has been reported to be 45 000 (ref. 22). More recently, Groth and Young[23] obtained a highly purified rat liver adenine phosphoribosyltransferase which has a molecular weight of 20 000, and showed that this purified enzyme contained a bound phosphoribosyl group. Our 4000-fold purified adenine phosphoribosyltransferase binds with *P*-Rib-*P*-*P* in the absence of Mg^{2+} to give a molecular species with a molecular weight of 26 000. In the absence of *P*-Rib-*P*-*P*, the elution pattern of adenine phosphoribosyltransferase shows catalytically active species with apparent molecular weights ranging from 18 000 to 45 000. Hochstadt-Ozer and Stadtman[24] have reported similar effects of Mg^{2+} on adenine phosphoribosyltransferase from *Escherichia coli* K-12.

Binding of *P*-Rib-*P*-*P* to adenine phosphoribosyltransferase in the absence of Mg^{2+} has also been reported by others[22,25,26].

P-Rib-*P*-*P* protects human adenine phosphoribosyltransferase against heat inactivation as shown previously for Ehrlich ascites tumor[26,27]. This appears to reflect firm bonds between *P*-Rib-*P*-*P* and adenine phosphoribosyltransferase which are not broken by filtration through Sephadex G-25. The change in physical properties of the enzyme induced by *P*-Rib-*P*-*P* were not associated with changes in antigenicity. However, chromatography on DEAE-cellulose restores heat lability to the Lesch-Nyhan enzyme[13].

It is not possible to establish unequivocally that the increased stability observed with *P*-Rib-*P*-*P* *in vitro* represents the same mechanism responsible for the prolonged survival of adenine phosphoribosyltransferase in Lesch–Nyhan erythrocytes *in vivo*, though it is a reasonable assumption[4,28,29]. The adenine phosphoribosyltransferase of some patients with hypoxanthine phosphoribosyltransferase 0.1% of normal is heat stable but the amount of adenine phosphoribosyltransferase in erythrocytes is normal[10]. Thus, heat stability and *in vivo* longevity may not be directly related.

It has been established in a number of systems that isozymes are specific in their antigenicity[6–8]. In the case of lactate dehydrogenase, anti-lactate dehydrogenase$_1$ does not cross react with lactate dehydrogenase$_5$ and *vice versa*. Also, lactate dehydrogenase$_1$ and lactate dehydrogenase$_5$ migrate differently in immunoelectrophoresis[30]. It has also been observed that anti-lactate dehydrogenase$_5$ does not inhibit the activity of lactate dehydrogenase$_1$, partially inhibits that of lactate dehydrogenase$_3$ and results in essentially complete inhibition of lactate dehydrogenase$_5$.

Immunological characterization of the adenine phosphoribosyltransferase enzyme in normal and Lesch–Nyhan erythrocytes was undertaken using immunoelectrophoresis and immunodiffusion on Ouchterlony plates and no differences could be detected. The ratio of antigenicity to catalytic activity was also determined and there was no difference between the Lesch–Nyhan and normal erythrocyte adenine phosphoribosyltransferase. These negative results provide indirect support of the thesis that the prolonged survival of adenine phosphoribosyltransferase in Lesch-Nyhan erythrocytes observed *in vivo* derives from extrinsic effectors rather than intrinsic changes in enzyme structure.

An incidental deduction can be made from the constancy of the ratio of antigenicity to catalytic activity. Since the ratio remains constant in spite of major differences in half-life, the rate of denaturation associated with aging must be the same for both antigenic and catalytic sites. Direct measurement supports this postulate (Yip, L. and Balis, M. E., unpublished). This would suggest that either the entire molecule is degraded or the sites are very close to each other. Rabbit antiserum prepared against human adenine phosphoribosyltransferase cross-reacts with erythrocyte lysate of monkey indicating structural similarities. This was not observed with lysates of lower animals. However, the amount of monkey enzyme activity neutralized by the anti-human adenine phosphoribosyltransferase is considerably greater than that observed with human enzyme indicating that while the enzymes appear to be similar, they are not identical. In preliminary studies with the related enzyme hypoxanthine phosphoribosyltransferase, a much different species pattern (Yip, L., Piomelli, S., Dancis, J. and Balis, M. E., unpublished) has been observed.

There is no known function for adenine phosphoribosyltransferase in spite of its

ubiquitous distribution except for lines specifically isolated as resistant to adenine analogues. The supposed substrate, adenine, is not ordinarily found in mammalian tissues, and no enzyme system which will lead to adenine production in man is known. However, it must be recalled that until Lesch–Nyhan disease was recognized hypoxanthine phosphoribosyltransferase was considered a useless "salvage enzyme".

ACKNOWLEDGEMENT

The authors wish to acknowledge the excellent technical assistance of Mrs Carol Gryte.

The authors also wish to thank the New York Blood Bank for their gift of outdated erythrocytes.

This investigation was supported by U.S. Public Health Service Research Grants CA 08748 and HD 04526 and by the National Foundation—March of Dimes. The reported patients were studied in the Special Clinical Unit of New York University Medical Center, New York, N.Y.

REFERENCES

1 Lesch, M. and Nyhan, W. L. (1964) *Am. J. Med.* 36, 561–570
2 Nyhan, W. L., Oliver, W. J. and Lesch, M. (1965) *J. Pediatr.* 66, 257–263
3 Seegmiller, J. E. Rosenbloom, F. M. and Kelley, W. N. (1967) *Science* 155, 1682–1683
4 Rubin, C. S., Balis, M. E., Piomelli, S., Berman, P. H. and Dancis, J. (1969) *J. Lab. Clin. Med.* 74, 732–741
5 Balis, M. E., Brown, G. F. and Cappucino, J. G. (1971) *Biochem. Biophys. Res. Commun.* 42, 1007–1011
6 Nisselbaum, J. S. and Bodansky, O. (1961) *J. Biol. Chem.* 236, 401–404
7 Eppenberger, H. M., Dawson, D. M .and Kaplan, N. D. (1967) *J. Biol. Chem.* 242, 204–209
8 Bigley, R. H., Stenzel, P., Jones, R. T., Campos, J. O. and Koler, R. D. (1968) *Enzymol. Biol. Clin.* 9, 10–20
9 Breitman, J. R. (1963) *Biochem. Biophys. Acta* 67, 153–155
10 Yü, T-F., Balis, M. E., Krenitsky, T. A., Dancis, J., Silvers, D. N., Elion, G. B. and Gutman, A. B. (1972) *Ann. Intern. Med.* 76, 255–264
11 Van Kampen, E. J. and Zylstra, W. G. (1961) *Clin. Chim. Acta* 6, 538–544
12 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275
13 Rubin, C. S. and Balis, M. E. (1972) *Biochim. Biophys. Acta* 279, 163–174
14 Rubin, C. S., Dancis, J., Yip, L. C., Nowinski, R. C. and Balis, M. E. (1971) *Proc. Natl. Acad. Sci. U.S.* 68, 1461–1464
15 Mancini, G., Carbonara, A. O. and Hereman, J. F. (1965) *Immunochemistry* 2, 235–254
16 Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* 121, 404–427
17 Balis, M. E., Krakoff, I. H., Berman, P. H. and Dancis, J. (1967) *Science* 156, 1122–1123
18 Horwitz, M. S. and Scharff, M. D. (1969) *Fundamental Techniques in Virology*, pp. 297–315, Academic Press, New York
19 Krenitsky, T. A. (1969) *Biochim. Biophys. Acta* 179, 506–509
20 Kornberg, A., Lieberman, L. and Simms, E. S. (1955) *J. Biol. Chem.* 215, 417–427
21 Hori, M. and Henderson, J. F. (1966) *J. Biol. Chem.* 241, 3404–3408
22 Berlin, R. D. (1969) *Arch. Biochem. Biophys.* 134, 120–129
23 Groth, D. P. and Young, L. G. (1971) *Biochem. Biophys. Res. Commun.* 43, 82–87
24 Hochstadt-Ozer, J. and Stadtman, E. R. (1971) *J. Biol. Chem.* 246, 5294–5303
25 Gadd, R. E. and Henderson, J. F. (1970) *J. Biol. Chem.* 245, 3404–3408
26 Hori, M. and Henderson, J. F. (1966) *J. Biol. Chem.* 245, 2979–2984
27 Murray, A. W. and Wong, D. C. L. (1967) *Biochem. J.* 104, 669–674
28 Greene, M. L., Boyle, J. A. and Seegmiller, J. E. (1970) *Science* 167, 887–889
29 Sorensen, L. B. (1970) *J. Clin. Invest.* 49, 968–978
30 Markert, C. L. and Appella, E. (1963) *Ann. N.Y. Acad. Sci.* 103, 915–929