Biochimica et Biophysica Acta, 568 (1979) 183—194

BBA 68743

PURIFICATION AND PARTIAL CHARACTERIZATION OF DIFFERENT FORMS OF PHOSPHOFRUCTOKINASE IN MAN *

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(Received October 20th, 1978)

Key words: Phosphofructokinase isoenzyme; (Properties, Human)

Summary

Phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) from human muscle, brain, heart and granulocytes has been purified using a two or three step purification procedure. The main step is Blue Dextran-Sepharose 4B chromatography with selective elution of phosphofructokinase by formation of the ternary complex ADP or ATP-fructose-6-*P*-enzyme.

Muscle and heart contain only enzyme subunits with a molecular weight of 85 000. This type of subunit is predominnant in brain, where it co-exists with subunits of about 80 000 daltons.

A single type of subunits is found in the granulocytes, with a molecular weight of 80 000.

Anti-muscle phosphofructokinase antiserum reacts only with M-type enzyme

Anti-granulocyte enzyme antiserum, absorbed by pure brain phosphofructokinase, exhibits a narrow specificity against the so-called L-type enzyme.

Anti-brain antiserum, absorbed by pure muscle phosphofructokinase and partly purified red cell enzyme, exhibits a narrow specificity against a phosphofructokinase form predominant in fibroblasts and present in brain (F-type).

Introduction

Human phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) has so far only been purified from muscle [1—3] and red

* This work was presented in part at the 12th FEBS Meeting, July 2—8, 1978, Dresden.

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cells [1,4]. It has been shown that the red cell enzyme is composed of two types of subunits, one similar to the muscle-type (or M-type), the only enzyme form in striated muscle and the other similar to the major form of liver (liver or L-type) [1,2,4—8].

In a recent paper [8], we proposed that a third basic phosphofructokinase form might exist in man, namely the F-type (i.e. fibroblast-type). This isozyme, mainly characterized by its electrophoretic and immunological features, was found in cultured fibroblasts (in which it represented the major form), platelets, lymphocytes and brain.

We attempted to develop convenient and rapid methods to produce the phosphofructokinase forms in a homogeneous state. The tissues used as starting material, were expected to contain one (or two) of the basic forms [8]: M-type in muscle, L-type in granulocytes, both M- and F-type in brain. We also purified heart phosphofructokinase, because some uncertainties exist as to its isozymic composition [8].

Materials and Methods

Materials

The substrates and auxiliary enzymes were from Boehringer-Mannheim (F.R.G.) or Sigma (St. Louis, MO, U.S.A.). Ion-exchange resins, CNBr-Sepharose 4B and Dextran Blue were supplied by Pharmacia (Uppsala, Sweden). Acrylamide, bisacrylamide, and sodium dodecyl sulphate (SDS) came from Eastman Kodak (Rochester, NY, U.S.A.). Polyethyleneglycol 6000 was from Fluka AG (Buchs, Switzerland). Enzyme activities were measured in a Gilford (Model 252) spectrophotometer. Absorption at 280 nm of the column eluates was measured with a Gilson apparatus (Model Holochrom). Ultrafiltration was performed with Amicon cell and membranes.

Methods

Assays. Enzyme activity of phosphofructokinase was assayed at 30°C in a 100 mM Tris-HCl buffer (pH 8) containing: 10 mM KCl, 5 mM $MgCl_2$, 5 mM $(NH_4)_2SO_4$, 1 mM EDTA, 5 mM dithiothreitol, 0.4 mg/ml bovine albumin, 0.2 mM NADH, 2 mM fructose-6-*P*, 0.5 I.U./ml aldolase, 0.4 I.U./ml glycerol-3-phosphate dehydrogenase and 10 I.U./ml triose-phosphate isomerase. After 10 min of preincubation at 30°C, reaction was started by adding ATP to the reaction mixture (0.5 mM for enzyme from granulocytes and 1 mM final concentration for enzyme from muscle, brain and heart). Protein concentrations was measured according to Lowry et al. [9], with bovine albumin as standard.

Tissue extraction. Granulocytes were isolated from a patient with hyperleukocytic acute myeloid leukemia as previously reported [10]. Striated muscle (quadriceps femoris and psoas), heart and brain were autopsy samples taken within 4 h after death and stored frozen (—80°C) until use. The tissues were homogenized at 0°C in a Potter-Elvehjem homogenizer, in 50 mM Tris-HCl buffer (pH 7.5)/10 mM $(NH_4)_2SO_4$/0.1 mM fructose-6-*P*/0.1 mM fructose-1,6-P_2/0.2 mM ATP/1 mM diisopropylphosphorofluoridate/2 mM ϵ-aminocaproic acid/10 mM dithiothreitol/2 mM EDTA/10 mM KF for brain and leukocytes, 30 mM KF for muscle and heart. The pH was immediately adjusted to pH 7.5 with solid Tris, then the extract was centrifuged for 20 min at

20 000 × *g*. In addition, the brain extract was treated with toluene in order to remove lipids [11].

$(NH_4)_2SO_4$ fractionation. The extracts were first precipitated by adding solid $(NH_4)_2SO_4$ (51 g/100 ml extract for muscle, 43 g/100 ml for brain and heart, 35 g/100 ml for granulocytes); pH was adjusted to 7.5 with solid Tris. The precipitates collected by centrifugation were washed at 4°C in $(NH_4)_2SO_4$ (buffered at pH 7.5 with solid Tris), 1 mM EDTA, 5 mM β-mercaptoethanol or dithiothreitol. The lower $(NH_4)_2SO_4$ concentration keeping the precipitated form was used (65% saturation for muscle, 55% for brain and heart, 45% for granulocytes). The precipitates were then partially dissolved in more diluted $(NH_4)_2SO_4$ solutions, so that the enzyme was completely recovered in the supernatant after centrifugation. These $(NH_4)_2SO_4$ solutions were 45% for muscle, 35% for brain and heart and 30% for granulocytes. $(NH_4)_2SO_4$ saturation of the active supernatants was raised with solid $(NH_4)_2SO_4$ in order to again precipitate the enzyme.

Chromatographies and buffers. DEAE-cellulose chromatography was used batchwise only for the brain extraction, according to the method of Layzer et al. [12].

The columns (2 × 20 cm) of Blue Dextran-Sepharose 4B were prepared according to Ryan and Vestling [13]. The basic buffer used (buffer A) was 20 mM Tris-HCl (pH 7.5)/10 mM $(NH_4)_2SO_4$/5 mM $MgCl_2$/10 mM NaF/2 mM ε-aminocaproic acid/10 mM dithiothreitol/1 mM EDTA/0.01 mM fructose-1,6-P_2. Ionic strength was then adjusted with different KCl concentrations for each type of tissue.

All the Blue Dextran-Sepharose chromatography experiments were carried out at 18°C.

Electrophoresis. SDS-polyacrylamide gel electrophoresis of the SDS-dissociated enzymes was performed according to Weber and Osborn [14] with RNA polymerase, phosphorylase, bovine albumin, catalase and aldolase as standards.

Immunization of rabbits, absorption of the antisera and purification of the immunoglobulins. Antisera were obtained by injecting pure enzyme from muscle, brain and granulocytes into rabbits. 500 μl for brain and muscle enzyme and 200 μl for granulocyte enzyme were emulsified with complete Freund's adjuvant, (1 vol. at 1 mg/ml enzyme solution + 1 vol. Freund's adjuvant), then injected into the back of the animals in multiple intradermal injections (0.1 ml/location); this procedure was repeated three times. The fourth and fifth injections were administred intramuscularly. The animals were killed 1 week after the last injection.

The antisera were tested, before absorption, by immunoprecipitation and (or) double immunodiffusion against extracts of muscle, heart, brain, granulocytes, liver, hepatoma, red cell and fibroblasts.

The absorption of the antisera was performed by incubating together antigen and antiserum for 1 h at 37°C and overnight at 4°C. After centrifugation for 30 min at 20 000 × *g*, the immunoglobulins were purified by two successive 40% $(NH_4)_2SO_4$ precipitations, followed by chromatography on DEAE-cellulose in 10 mM phosphate buffer (pH 6.8). The immunoglobulins were readily eluted in the excluded peak, concentrated by ultrafiltration on PM 30 membranes to a protein concentration of 5 mg/ml, and stored frozen at −80°C. Anti-muscle

phosphofructokinase antiserum was absorbed with enzyme from human hepatoma (this tumor contains both L- and F-type phosphofructokinase [8]. The enzyme was partially purified by $(NH_4)_2SO_4$ fractionation and DEAE-cellulose chromatography, then 5 I.U. phosphofructokinase were added/ml antiserum. Anti-granulocyte phosphofructokinase antiserum was absorbed with pure brain enzyme (4 I.U. enzyme/ml antiserum).

Anti-brain phosphofructokinase was absorbed by a mixture of 20 I.U./ml pure muscle enzyme and 10 I.U./ml partially purified red cell enzyme. Red cell phosphofructokinase was partially purified by DEAE-cellulose chromatography and $(NH_4)_2SO_4$ fractionation.

The absorbed immunoglobulins will be designated as anti M-type, anti L-type and anti F-type antisera.

Immunoprecipitation tests. The immunoprecipitation tests were performed as follow: the enzyme samples were mixed with increasing concentrations of antiserum or immunoglobulins in a 50 mM Tris/phosphate buffer (pH 8)/20 mM $(NH_4)_2SO_4$/10 mM NaF/10 mM EDTA/0.1 mM dithiothreitol/10 mM ϵ-aminocaproic acid/1 mM diisopropylfluorophosphate/0.1 mM fructose-6-*P*/ 0.1 mM fructose-1,6-P_2/0.2 mM ATP/1 mM AMP/1 mg/ml bovine serum albumin. In this incubation mixture, diisopropylfluorophosphate and ϵ-amino-caproic acid [15] were added as antiproteolytic agents. After incubation for 1 h at 37°C and overnight at 4°C polyethyleneglycol 6000 was added to each tube (to a final concentration of 7%, w/v); after 10 min at 4°C, the tubes were centrifuged for 15 min at 35 000 × *g*, and residual activity was measured in the supernatant. Polyethyleneglycol was used because of its ability to precipitate the soluble antigen-antibody complexes [16].

Results

Purification of muscle phosphofructokinase (Table I)

After extraction the fraction precipitating between 45—65% $(NH_4)_2SO_4$ saturation was collected, then desalted on a Sephadex G-25 column equilibrated with buffer A. The preparation was fixed on Blue Dextran-Sepharose equilibrated with buffer A. The column was then washed with this buffer +30 mM KCl until all absorbance at 280 nm disappeared. At that time,

TABLE I

PURIFICATION OF MUSCLE PHOSPHOFRUCTOKINASE

	Activity (I.U.)	Protein (mg)	Specific activity (I.U./mg)	Cumulative purification (-fold)	Yield (%)
Crude extract	4000	10 000	0.4	0	100
$(NH_4)_2SO_4$ fractionation	4800	1 200	4	10	120
Sephadex G-25 chromatography + Blue Dextran-Sepharose chromatography and elective elution	4150	21	198	495	104

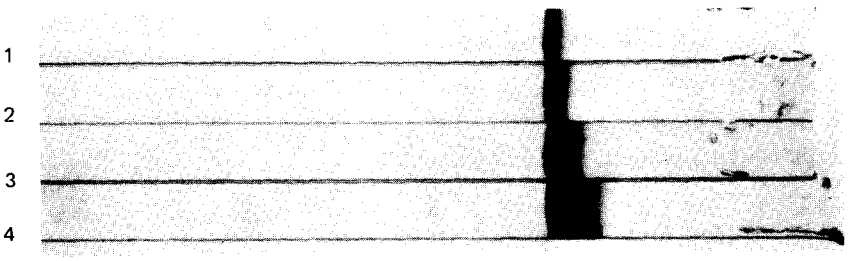

Fig. 1. SDS-polyacrylamide disc gel electrophoresis of muscle phosphofructokinase. 1, 10 μg protein; 2, 20 μg; 3, 50 μg; 4, 90 μg. Staining with amidoblack.

0.1 mM ADP was added to the washing buffer, until a linear absorbance line at 280 nm was obtained. The addition of 1 mM fructose-6-*P* to this buffer provoked elution of phosphofructokinase as a sharp peak. The enzyme was concentrated by ultrafiltration and stored frozen at −80° C as a precipitate in a 80% satd. $(NH_4)_2SO_4$. The yield of this two step procedure was apparently higher than 100% because of the initial reactivation of phosphofructokinase during $(NH_4)_2SO_4$ fractionation. The enzyme was purified about 500-fold and specific activity was 198 I.U./mg protein. A single band, without visible contaminant, was observed by SDS-polyacrylamide gel electrophoresis until a load of 90 μg protein/gel (5.5 × 110 mm). Molecular weight, determined from five independent experiments, was 85 500 ± 3100 (mean of five independant experiments ± 1 S.D.) (Figs. 1 and 3).

Purification of heart phosphofructokinase (Table II)

After extraction and $(NH_4)_2SO_4$ fractionation (the 35—55% fraction was collected), the preparation was desalted and applied to a column of Blue Dextran-Sepharose equilibrated with buffer A. The enzyme was eluted by buffer A + 100 mM KCl + 1 mM fructose-6-*P* + 0.1 mM ATP. The active frac-

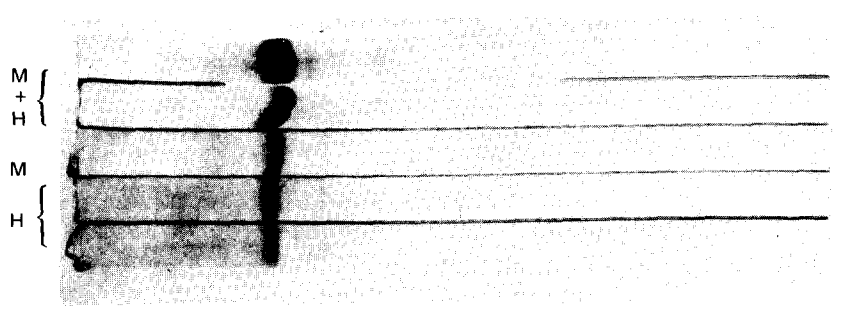

Fig. 2. SDS-polyacrylamide gel electrophoresis of heart and muscle phosphofructokinase. H, heart enzyme, 5 μg (left) and 10 μg (right); M, muscle enzyme, 5 μg; H + M, mixture of heart and muscle phosphofructokinase, 10 μg (left) and 20 μg (right). Staining with Coomassie blue.

TABLE II

PURIFICATION OF HEART PHOSPHOFRUCTOKINASE

	Activity (I.U.)	Protein (mg)	Specific activity (I.U./mg)	Cumulative purification (-fold)	Yield (%)
Crude extract	1190	31 315	0.038	1	100
$(NH_4)_2SO_4$ fractionation	900	1 667	0.54	14.2	76
Sephadex G-25 chromatography + first Blue Dextran-Sepharose chromatography $(NH_4)_2SO_4$ precipitation	600	30	20	526	50
Second Blue Dextran-Sepharose chromatography and elective elution					
Fraction 1 (pure)	448	4.85	92	2431	38
Fraction 2 (not pure)	100				8

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tions were precipitated by $(NH_4)_2SO_4$, desalted, then applied again to a Blue Dextran-Sepharose column (1 × 6 cm). The major part of the heart enzyme (fraction 1) was eluted under the same conditions as the muscle enzyme. A second fraction was eluted at higher KCl content (100 mM). This fraction (fraction 2), was not pure. Fraction 1 had a specific activity of 92 I.U./mg and, upon SDS-polyacrylamide gel electrophoresis, migrated as a single band, without any visible contaminant up to a load of 20 μg protein. With a higher load, two faint bands were visible in a region corresponding to low molecular weight (20 000—30 000). The molecular weight of the subunits of heart phosphofructokinase (fraction 1) was similar to that of the muscle-type subunits (i.e. about of 85 000) (Fig. 2).

Purification of granulocyte phosphofructokinase (Table III)

Granulocyte enzyme was precipitated between 30—45% $(NH_4)_2SO_4$ saturation. The precipitate was desalted on a Sephadex G-25 column equilibrated with buffer A + 30 mM KCl, then the extract was adsorbed onto a Blue Dextran-Sepharose column equilibrated against the same buffer. The column was washed with buffer A + 70 mM KCl until absorbance at 280 nm was zero. Then 0.05 mM ATP was added to this buffer. When the absorbance line was linear, phosphofructokinase elution was initiated by addition of 1 mM fructose-6-*P*. The elution peak, in contrast with that obtained with muscle-type phos-

TABLE III

PURIFICATION OF PHOSPHOFRUCTOKINASE FROM LEUKEMIC MYELOBLASTS

	Activity (I.U.)	Protein (mg)	Specific activity (I.U./mg)	Cumulative purification (-fold)	Yield (%)
Crude extract	692	6291	0.11	0	100
$(NH_4)_2SO_4$ fractionation	535	892	0.6	5.4	77
Sephadex G-25 chromatography + Blue Dextran-Sepharose chromatography and elective elution	360	4.2	85	773	52

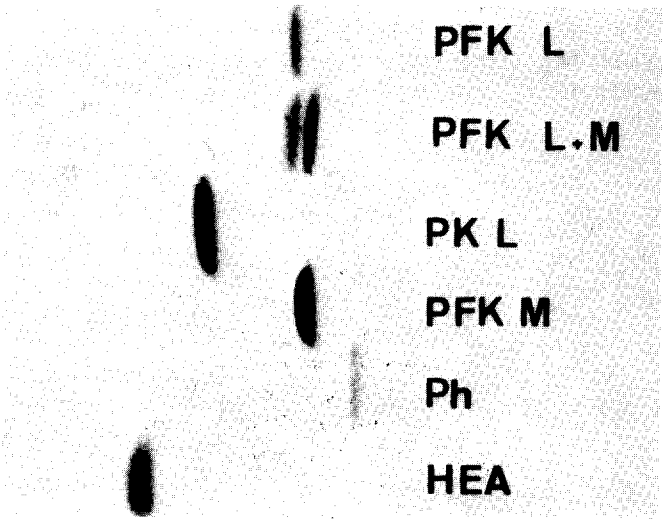

Fig. 3. SDS-polyacrylamide slab gel electrophoresis. PFK L, granulocyte enzyme, 10 μg; PFK L + M, mixture of 10 μg granulocyte enzyme and 20 μg of muscle enzyme; PK L, human liver L-type pyruvate kinase 20 μg, PFK M, muscle phosphofructokinase, 20 μg; Ph, rabbit muscle phosphorylase, 5 μg; HEA. hen egg albumin, 10 μg. Staining with amidoblack.

phofructokinase, was not symmetrical. Phosphofructokinase was 773-fold purified, with a yield of 52%; the specific activity of the purified preparation was 85 I.U./mg protein. In SDS-polyacrylamide gel electrophoresis a single protein band was seen with protein loads lower than 30—40 μg; this band corresponded to subunits with molecular weight 79 500 ± 3600 (mean of five independent experiments (±1 S.D.) (Fig. 3).

Purification of brain phosphofructokinase (Table IV)

Using brain extracts, it was essential to eliminate the lipids from the extract by a toluene extraction before beginning purification. The first step was, batchwise, chromatography on DEAE-cellulose [12]. The active eluate was fractionated by $(NH_4)_2SO_4$, with the enzyme precipitating between 35—55%

TABLE IV

BRAIN PHOSPHOFRUCTOKINASE PURIFICATION

	Activity (I.U.)	Protein (mg)	Specific activity (I.U./mg)	Cumulative purification (-fold)	Yield (%)
Crude extract, toluene treatment	1800	33 962	0.053	0	100
DEAE-cellulose chromatography	1500	2 206	0.68	12.8	83
$(NH_4)_2SO_4$ fractionation	1200	1 000	1.2	22.6	67
Sephadex G-25 chromatography + Blue Dextran-Sepharose chromatography and elective elution					
Fraction 1	350	5	70	1320	50
Fraction 2	585	8.6	68	1283	

saturation. Then, after desalting on Sephadex G-25 column, the preparation was applied to a Blue Dextran-Sepharose column as described for muscle. A first fraction was eluted in the same conditions as the muscle enzyme ('brain fraction 1').

A second fraction was eluted at higher ionic strength: after elution by ADP-fructose-6-*P* of the fraction the column was washed with buffer A + 60 mM KCl. After addition of 0.05 mM ATP, elution was initiated by 1 mM fructose-6-*P*. The resulting peak (fraction 2) was not symmetrical. Fraction 1 yielded a single band in SDS-polyacrylamide gel, with similar molecular weight to that of the the muscle-type subunits. Fraction 2 yielded a major band migrating similar to the muscle enzyme, and a minor band migrating similar to the granulocyte enzyme. When the protein load was higher than 20 μg/gel, faint supplementary bands were observed in fraction 2. The overall yield of this procedure was 50%, specific activity being about 70 I.U./mg for both fractions; this represented a 1300-fold purification. The molecular weights of the different phosphofructokinase subunits are summarized in Table V.

Immunodiffusion tests. Anti-granulocyte phosphofructokinase antiserum yielded a single precipitin line with crude extracts of polymorphonuclear cells, testis, liver, hepatoma and red cells (Fig. 4). All these lines showed a pattern of immunological identity; they could be stained specifically for phosphofructokinase activity. This antiserum did not react with muscle enzyme, neither with the major fraction of heart enzyme. By contrast, a faint precipitin line was observed with heart fraction 2 (Fig. 4). Fig. 5 shows that anti-muscle phosphofructokinase antiserum gave a single precipitin line with muscle (crude extract or pure preparation) and heart enzyme. A faint line was also observed with partially purified red cell preparation, but not with polymorphonuclear cell extract. A spur could be observed between the lines corresponding to muscle on the one hand, red cells and heart on the other. Neither brain sample nor anti-brain enzyme antiserum were available for this study which explains the absence of reference to this tissue in Figs. 4 and 5.

Immunoprecipitation tests. We studied residual enzyme activity after precipitation by increasing amounts of antiserum or purified immunoglobulins. It is therefore possible to plot a 'precipitation curve', which reaches a low

TABLE V

ANALYSIS OF THE SUBUNIT COMPOSITION OF THE DIFFERENT PHOSPHOFRUCTOKINASES BY SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS

The values given for muscle and granulocytes are the mean of five different measures ± S.D. The difference between molecular weight of muscle and granulocyte phosphofructokinase is significant for $P < 0.01$ (Student's *t*-test).

	Molecular weight of the SDS-dissociated subunits
Muscle	85 000 ± 3100 (n = 5)
Granulocytes	79 500 ± 3600 (n = 5)
Brain	
1	≈85 000
2	major: ≈85 000; minor: ≈80 000
Heart 1	≈85 000

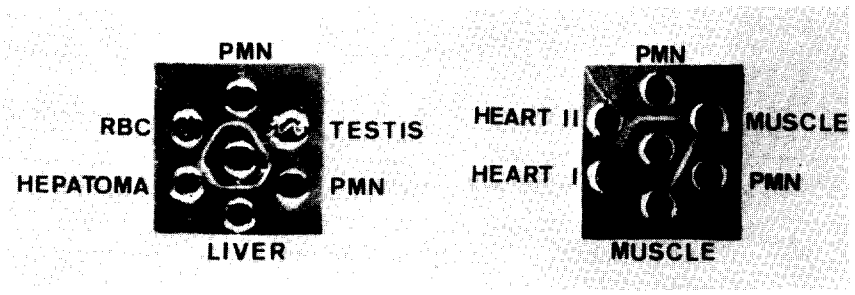

Fig. 4. Double immunodiffusion analysis using crude anti-granulocyte phosphofructokinase antiserum. Antiserum was put in the inner wells, antigen in the outer wells. PMN, mature polymorphonuclear cells; RBC, red blood cells; Heart 1, heart fraction 1; heart 2, heart fraction 2.

horizontal plateau representing residual enzyme activity after maximum precipitation of the enzyme. The results of this maximum precipitation (expressed as a percentage of the initial activity) are given in Table VI.

Anti-muscle phosphofructokinase antiserum was, even before absorption, practically monospecific for the M-type enzyme: it reacted with phosphofructokinase from muscle, heart, brain and red cells, but not with enzyme from leukocytes and fibroblasts.

By contrast, anti-granulocyte phosphofructokinase antiserum was, before absorption by brain phosphofructokinase, mainly active on L-type enzyme, slightly active on F-type enzyme and inactive on M-type enzyme. It neutralized totally phosphofructokinase from leukocytes, partially enzyme from brain (mainly fraction 2), fibroblasts and red cells. It was without any effect on

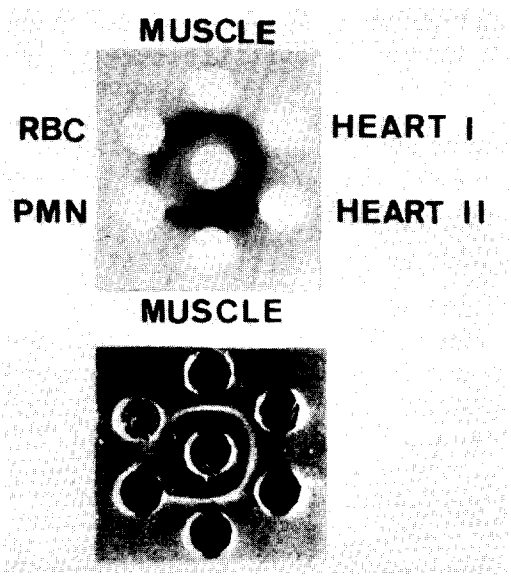

Fig. 5. Double immunodiffusion analysis using crude anti-muscle phosphofructokinase antiserum. In the upper picture the immunoprecipitin lines were stained with amidoblack.

TABLE VI

MAXIMUM IMMUNOPRECIPITATION OF PHOSPHOFRUCTOKINASE FROM VARIOUS TISSUES BY THE CRUDE AND ABSORBED ANTISERA

Anti M, anti L and anti F immunoglobulins (Ig) refer to the absorbed antisera whose immunoglobulins have been purified.

	% of residual activity after precipitation by excess anti-phosphofructokinase antisera					
	Anti-muscle		Anti-granulocytes		Anti-brain	
	Crude	Anti M Ig	Crude	Anti M Ig	Crude	Anti F Ig
Muscle *,**	<10	<10	100	100	<10	80—100
Granulocytes *	100	100	<10	<10	20	60
Brain *						
1	<10	<10	90	100	<10	90
2	<10	<10	50	100	<10	50
Heart *						
1	<10	<10	100	100	<10	80—100
2	<10	<10	80—90	100	<10	80—90
Fibroblasts **	80—100	100	30	55—79	<10	15—25
Polymorphonuclear cells **	100	100	<10	<10	20—30	70—85
Red cells **	45—55	45—55	20—35	20—35	<10	65—80

* Pure preparations.
** Homogenates.

muscle and heart enzymes. After absorption by brain phosphofructokinase, this antiserum became practically specific for L-type enzyme: neutralization of the tissues containing F-type phosphofructokinase disappeared or strongly decreased (e.g. brain and fibroblasts).

Anti-brain phosphofructokinase, before absorption, neutralized enzyme from all the tissues and preparations studied. After absorption by muscle and red cell phosphofructokinase (this latter enzyme containing L- and M-type subunits) this antiserum reacted mainly with the tissues and preparations containing F-type subunits (brain and fibroblasts). A very slight neutralization was also observed with tissues containing mainly (or exclusively) the L-type isozymes (polymorphonuclear cells and red cells). Pure granulocyte phosphofructokinase from leukemic cells was neutralized by 40%.

Discussion

Purification of muscle, brain and heart phosphofructokinase has already been performed from various sources and using various procedures [1—4, 17—19]. The methods we propose are probably among those, so far published, which are the most rapid and easy: in only two or three steps, and two or three days, they enable the purification of the enzymes to a good degree of purity, with a very high yield. As for phosphofructokinase from leukocytes, its total purification is reported for the first time.

Neither Blue Dextran-Sepharose chromatography [20,21], nor specific elution by formation of the ternary complex ATP or ADP-fructose 6-*P*-enzyme [3] were individually original methods; this type of specific elution, however, is

used for the first time in association with Blue Dextran-Sepharose chromatography, and is proven to work very well in these conditions. Since Blue Dextran-Sepharose is by far less tedious or less expensive to prepare than ATP-Sepharose [3], these techniques should probably be used by all the scientists working with mammal phosphofructokinase. Just a point is to be noted concerning the use of Blue Dextran-Sepharose: the properties of each batch of the absorbent may slightly differ, and the exact elution conditions are to be determined in each case. Since each column is easily regenerated using 8 M urea + 2 M NaCl, a same batch can be used for a long period using the same conditions.

We are able to confirm in this paper molecular weights of the M-type and L-type subunits, in particular studied from red cells enzyme [1,2]. We also confirm that M-type subunits are predominant in heart and brain, as this was proposed from studies of other mammals as man [22,23]. However, if enzyme from muscle and major form from heart and brain exhibit identical molecular weight and closely related antigenicity, they differ in several points: their electrophoretic mobility is not the same, whatever electrophoretic medium is used [8]; specific activity of the pure enzymes varied from 68 (brain, fraction 1) to 198 (muscle). In addition heart and muscle enzymes give, in double immunodiffusion, lines forming a 'spur', which could indicate some antigenic differences between them. Rather than to hypothesize that three distinct genes could code for phosphofructokinase in muscle, heart and brain, we prefer to speculate that these differences could be consequences of postsynthetic modifications whose the nature and the mechanism remain to be elucidated.

In addition to M-type subunits, brain enzyme contains another minor type of subunits whose molecular weight is approx. 80 000; immunologically some F-type and L-type enzymes are detected. Leukemic granulocytes enzyme shows a single band by SDS-polyacrylamide gel electrophoresis, corresponding to molecular weight about of 80 000. Immunologically this enzyme seems to be composed mainly of L-type phosphofructokinase, and slightly reacts with anti F-type antibodies. The antisera obtained by injecting this enzyme to rabbits exhibits a strong anti L-type, and a slight anti F-type specificity. This latter specificity is absorbed by brain phosphofructokinase.

It is impossible to ascribe with certainty a molecular weight to the F-type subunits from these results *. This will be only done by purifying a phosphofructokinase form composed only of F subunits. Such a work is now in progress in our laboratory. It can only be speculated that the molecular weight of the F subunits should be close to 80 000 or 85 000 since these values correspond to the two bands observed in the brain preparation. In the latter case the slight F-type immunological reactivity observed for granulocyte phosphofructokinase could be explained by a certain cross-reactivity between L and F subunits.

In conclusion this paper describes a rapid and convenient method enabling the purification of the different forms of human phosphofructokinase. From the enzymes purified from muscle, granulocytes and brain it was possible to obtain antisera which, after absorption, exhibited three distinct specificities,

* See Note added in proof.

namely anti M-type, anti L-type and anti F-type. These specificities are expected to correspond to the three basic forms of the enzyme. The M-type subunits have molecular weight of 85 000, the L-type of 80 000 while molecular weight of the F subunits remains undetermined. Finally it is hypothesized that the differences between M-type enzyme from muscle, heart and brain could be partly explained by postsynthetics events.

Note added in proof (Received 28th February, 1979)

(1) Since this paper was written we have found that the best buffer to be used at the step of Blue Dextran Sepharose 4 B chromatography is a 20 mM Tris-phosphate buffer (pH 7.5)/10 mM KF/10 mM $(NH_4)_2SO_4$/0.01 mM fructose $1.6P_2$/0.1 mM EDTA/10 mM dithiothreitol. Elution is always provoked by 0.05 mM ADP, 1 mM fructose 6-P in the presence of various KCl concentration according to the forms to be purified: 30 mM for M_4 enzyme, 80 mM for M-L hybrids, 250 mM for F_4 and L_4.

(2) F_4 has been purified from platelets; molecular weight of the F subunits is 85 000—87 000 (Kahn et al., unpublished results).

Acknowledgements

This work was supported in part by grants from INSERM (CRL 78.5.125.1 and ATP 56.78.88). We are grateful to Mrs. M. Urbanek and Ms. F. Carrouget for typing the manuscript. We also thank R. Kernemp for his excellent technical assistance.

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