Biochimica et Biophysica Acta, 611 (1980) 114—126

BBA 68873

PURIFICATION OF F_4 PHOSPHOFRUCTOKINASE FROM HUMAN PLATELETS AND COMPARISON WITH THE OTHER PHOSPHOFRUCTOKINASE FORMS

AXEL KAHN *, DOMINIQUE COTTREAU and MARIE CLAIRE MEIENHOFER

Institut de Pathologie Moléculaire, INSERM U 129, Chu Cochin, 75674 Paris Cedex 14 (France)

(Received May 10th, 1979)

Key words: F-subunit; Phosphofructokinase isoenzyme; (Platelet)

Summary

The phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) tetramers F_4, F_3L and F_2L_2 have been separated from human platelets, and purified to homogeneity by affinity chromatography on Dextran Blue-Sepharose 4B.

The F subunits have a molecular weight of 85 000, identical to that of the M subunits. By contrast with L-type phosphofructokinase, the F-type enzyme seems to exist predominantly in a tetrameric form and not to aggregate to high molecular weight polymers. Specific activity of pure F_4 phosphofructokinase is about 140 IU/mg of protein.

Immunologically, it is easy to distinguish all the basic phosphofructokinase forms (i.e. M, L and F types); nevertheless a slight immunological cross-reactivity seems to exist between all these forms.

Introduction

In previous reports we have shown that heterogeneity of the different phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) forms in man could be explained on the basis of three fundamental subunits called M (muscle type), L (liver type) and F (fibroblast type). These subunits are expected to correspond to three distinct genetic loci [1,2].

M_4 enzyme was purified from muscle, brain and heart, and L_4 from leukemic granulocytes [1,3]. The molecular weight of the L and M subunits was about

* To whom reprint requests should be sent.

80 000 and 85 000, respectively. F subunits were antigenically detected in brain [1,2], but we were unable to determine their molecular weight with certainty.

Hence, this work was undertaken for further characterization of F subunits of human phosphofructokinase which were purified to homogeneity from platelets.

Materials and Methods

Materials

Special chemicals and enzymes were obtained from Sigma (St. Louis, MO, U.S.A.), Boehringer-Mannheim (F.R.G.) and Serva (Heidelberg, F.R.G.). Common chemicals were standard reagent grade. DEAE-cellulose (DE52) was supplied by Whatman (Springfield, U.K.); CNBr-activated Sepharose 4B and Dextran Blue were obtained from Pharmacia (Uppsala, Sweden). Acrylamide, bisacrylamide and sodium dodecyl sulfate (SDS) came from Eastman Kodak (Rochester, NY, U.S.A.). Polyethyleneglycol 6000 was from Fluka AG (Buchs, Switzerland). Cellulose acetate plates were from Helena (Beaumont, TX, U.S.A.). Agarose ('Indubiose') was produced by 'l'Industrie Biologique Française' (Lyon, France). SDS-polyacrylamide gradient slab gels were prepared in the laboratory using an 'Uniscil' gradient former (Universal Scientific Limited, London, U.K.), and the electrophoreses in polyacrylamide gels were performed in the electrophoresis apparatus furnished by the same company. For the migrations in non-dissociating conditions, preformed 2.7–24% polyacrylamide (w/v) gradient gels 'gradipore' were used (Uniscil, London, U.K.).

Enzyme activities were measured in a Gilford (Model 252) spectrophotometer. Absorption at 280 nm of the column eluates was measured with a Gilson apparatus.

Methods

Cell fractionation, extraction and enzyme assays. Outdated platelets were obtained from blood banks. Most of the contaminant leukocytes and red cells were eliminated by a 15 min centrifugation at 330 × *g* and 4°C. The remaining erythrocytes were specifically lysed by suspending the cell pellet (obtained by a 20 min centrifugation at 2400 × *g*) in 0.15 M NH_4Cl (20 min at room temperature). The platelets were three times washed in 0.15 M NaCl/10 mM EDTA (pH 6.4). Extraction was performed in a 5 mM Tris/phosphate buffer (pH 7.5)/4 mM $(NH_4)_2SO_4$/10 mM dithiothreitol/0.1 mM EDTA/0.2 mM ATP/0.1 mM fructose-6-*P*/0.1 mM fructose-1,6-P_2/1 mM diisopropylphosphorofluoridate.

After homogenization at 4°C for 1 min in a Waring blendor the homogenate was adjusted to pH 7.5 with solid Tris, then centrifuged for 30 min at 20 000 × *g*. The supernatant was then treated with toluene to remove lipids [4].

In one experiment (whose the purpose was to determine whether partial proteolysis of phosphofructokinase could occur during cell extraction and enzyme purification), homogenization of the platelets was performed in the presence of a mixture of several antiproteolytic substances composed, in addi-

tion to diisopropylphosphorofluoridate, of 1 mM phenylmethylsulfonyl fluoride, 0.5 mM *N*-α-*p*-tosyllysine chloromethylketone, 0.5 mM *p*-tosylamido-2-phenylethyl chloromethylketone, 0.05 mM cathepsin D inhibitor pepstatin [5] and 2.5 μg/ml cathepsin B inhibitor leupeptin [6].

Enzyme activity of phosphofructokinase was assayed at 30°C as reported elsewhere [1—3].

Purification of phosphofructokinase. $(NH_4)_2SO_4$ fractionation was performed as described in a previous paper [1].

For all the chromatographies a same buffer (called buffer A) was used: it was a 20 mM Tris/phosphate buffer (pH 7.5)/10 mM $(NH_4)_2SO_4$/10 mM KF/10 mM dithiothreitol/0.1 mM EDTA/0.01 mM fructose-1,6-P_2/1 mM diisopropylphosphorofluoridate. All the DEAE-cellulose, Sephadex G-25 and Dextran Blue-Sepharose columns were equilibrated with it. The chromatographies on DEAE-cellulose were carried out at 18°C, the other chromatographies being performed at 4°C.

Electrophoreses. Electrophoresis on cellulose acetate. The samples were dialysed against the electrophoretic buffer, then applied to the cellulose acetate plates using the special applicator furnished by the manufacturer (this apparatus enables the simultaneous applications of eight samples of 2 μl each). The electrophoretic buffer (in which the plates were equilibrated before application of the samples) was a 5 mM Tris/maleate buffer (pH 7.2)/5 mM $(NH_4)_2SO_4$/0.5 mM ATP/0.5 mM $MgCl_2$/0.1 mM dithiothreitol/0.01 mM fructose-1,6-P_2/0.1 mM EDTA. Electrophoresis was carried out for 45 min at 4°C, at 35 V/cm: migration was towards the anode. Phosphofructokinase activity was revealed by pouring onto the gel a layer of 1% agarose containing 100 mM Tris-HCl (pH 8)/5 mM $(NH_4)_2SO_4$/5 mM $MgCl_2$/1 mM NAD^+/10 mM sodium arsenate/1 mM sodium pyruvate/0.5 mM ATP/1 mM fructose-6-*P*/0.5 IU/ml aldolase/0.5 IU/ml glyceraldehyde-3-phosphate dehydrogenase/0.4 mg/ml nitroblue tetrazolium and 0.02 mg/ml phenazine methosulfate. The enzymic bands appeared after about 1 h in the dark at 37°C. Specificity of the staining for phosphofructokinase activity was checked by omitting either fructose-6-*P* or ATP from the staining mixture.

Polyacrylamide gradient slab gel electrophoresis. The preformed gels (2.7—24% acrylamide, w/v) were first equilibrated against the 'tank buffer' by a prerun of 2 h with a current of 30 mA/plate (2 × 80 mm). Then, after application of the samples (about 5 μg of protein/sample), migration was continued for 18 h with a current of 30 mA/plate, at a temperature of 2—4°C. The tank buffer was 20 mM Tris/glycine (pH 8.9)/3 mM K_2HPO_4/2 mM $(NH_4)_2SO_4$/0.2 mM ATP/0.1 mM fructose-1,6-P_2/0.1 mM dithiothreitol. After the migration was stopped, the gels were sliced into two slices; one of them was stained for proteins with Coomassie brilliant blue R 250 [7] and the other was stained for phosphofructokinase activity, as described for the cellulose acetate electrophoresis.

SDS-polyacrylamide gradient slab gel electrophoresis. Electrophoresis in SDS-polyacrylamide slab gel was performed according to Laemli [8], except that the running gel was a 9—24% (w/v) polyacrylamide gradient gel. Migration was continued for 15 h at 20 mA/plate. After the migration was stopped, the proteins were fixed and SDS was eliminated by a continuous agitation in a 50%

(v/v) methanol/10% (v/v) acetic acid solution changed each 2 h for 8 h. Proteins were stained with Coomassie blue [7].

Immunological characterization. Preparation of the specific antisera directed against the M, L and F phosphofructokinase subunits has been previously described [1,2]. A new anti-F-type antiserum, with a higher titer than that previously described [1], was used in this study. This antiserum (called anti-F_2) was raised in rabbits by injecting them a purified human kidney enzyme preparation. Kidney enzyme is composed of F-type subunits as major form and L and M-type subunits as minor forms [2]. After absorption with muscle and erythrocyte phosphofructokinase, this antiserum became specific for the F-type enzyme and the F-containing hybrids. Immunoglobulins were purified as described elsewhere [1—3].

The methods of immunoprecipitation and double immunodiffusion used in this study have been previously reported [1—3].

Results

Separation of the different tetrameric forms of platelet phosphofructokinase

After lysis of the cells and toluene extraction, the crude extract was mixed with DEAE-cellulose (DE52) previously equilibrated with buffer A (about 100 ml of moist resin/200 IU of enzyme activity). The ion exchanger was gathered in a Büchner funnel and washed with buffer A (about 1 l of buffer/100 ml resin). Phosphofructokinase was eluted with buffer A containing 200 mM $(NH_4)_2SO_4$, 100 mM KH_2PO_4, adjusted to pH 7. The enzyme was then fractionated by $(NH_4)_2SO_4$ precipitation as previously reported [1]; it was precipitated between 30 and 50% saturation.

The precipitate was collected by centrifugation, dissolved in buffer A, desalted on a Sephadex G-25 column equilibrated with buffer A, then applied to a DEAE-cellulose column (3.5 × 20 cm) also equilibrated with buffer A. Phosphofructokinase was eluted by a linear gradient between 1200 ml of buffer A and 1200 ml of buffer A containing 100 mM $(NH_4)_2SO_4$, 100 mM KH_2PO_4, adjusted at pH 7. Three activity peaks were separated (Fig. 1) and precipitated by solid $(NH_4)_2SO_4$ (35 g/100 ml).

Characterization of the phosphofructokinase peaks

Fig. 2 shows that each peak separated by DEAE-cellulose chromatography corresponded to an electrophoretically distinguishable form. Fibroblast and mature polymorphonuclear cell extracts were applied on the same cellulose acetate strip as the platelet enzymes; they enabled to locate the F_4 and L_4 forms which have been proved to be the predominant forms in these cells [2,3]. From the electrophoretic positions of phosphofructokinase from peaks 1—3, it could be assumed that they corresponded to the tetrameric forms F_4, F_3L and F_2L_2, respectively. The slight difference of electrophoretic pattern between platelet peak 1 and fibroblast enzyme could be due to the use of a partially purified preparation for the former sample and of a crude extract for the latter; it is not noted if partially purified fibroblast phosphofructokinase is used (not shown).

Immunological characterization of phosphofructokinase of these peak was in

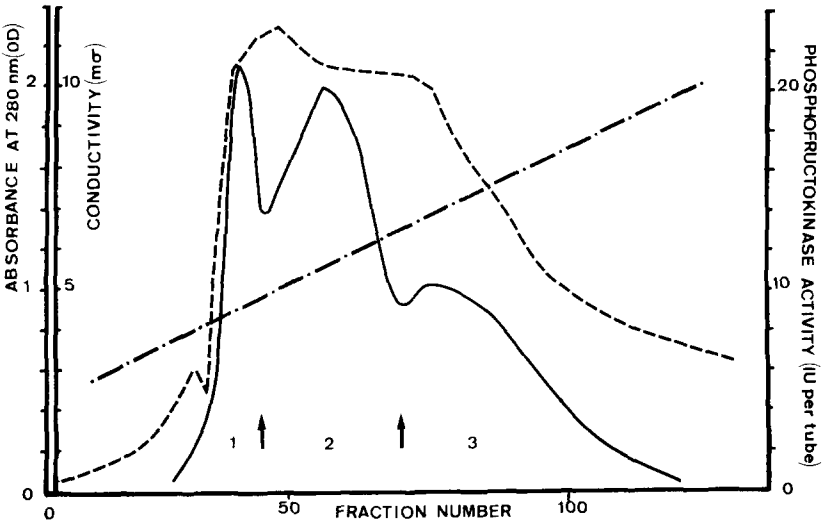

Fig. 1. Elution profile of platelet phosphofructokinase from DEAE-cellulose column. ———, enzyme activity; - - - - - -, absorbance at 280 nm; — · — · —, ionic strength. The arrows indicate the limit of the peaks 1—3.

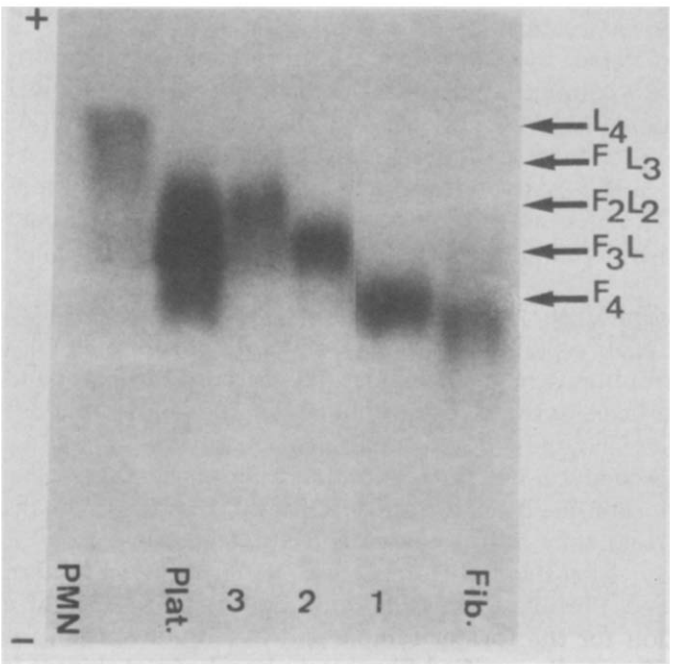

Fig. 2. Electrophoresis on cellulose acetate. Staining for phosphofructokinase activity. Fib, fibroblasts; 1, platelet enzyme, peak 1; 2, platelet enzyme, peak 2; 3, platelet enzyme, peak 3; plat, platelet crude extract; PMN, polymorphonuclear cell extract.

total agreement with this assumption (Table I). Anti-M-type antiserum was practically inactive on each fraction. Anti-L-type antiserum poorly neutralized the enzyme in peak 1, and increasingly in peaks 2 and 3. Anti-F-type antiserum was very active on each fraction.

Affinity chromatography on Dextran Blue-Sepharose 4B columns

Phosphofructokinases from peaks 1 (expected to correspond mainly to the F_4 enzyme) and mixed peaks 2 and 3 (isozymes F_3L and F_2L_2) were separately purified. The preparations were desalted by chromatography on Sephadex G-25, then applied to Dextran Blue-Sepharose 4B columns [1,2] equilibrated with buffer A. 10 ml of packed absorbent are able to fix more than 200 IU of phosphofructokinase.

The columns were washed with buffer A + 220 mM KCl and 0.1 mM $MgCl_2$ untill all absorbance at 280 nm has disappeared. Elution of phosphofructokinase was obtained with buffer A + 200 mM KCl, 0.1 mM $MgCl_2$, 0.05 mM ADPand 1 mM fructose-6-*P*.

The active eluate was concentrated by ultrafiltration on Amicon membranes (PM 30), then precipitated with solid $(NH_4)_2SO_4$ (35 g/100 ml). The enzyme was stored frozen at −80°C under the form of a $(NH_4)_2SO_4$ precipitate, protein concentration being about 2 mg/ml.

This procedure yielded enzymes with specific activity of 140 IU/mg of protein for peak 1 and 110 IU/mg for peaks 2 and 3, which corresponded to a 3700 and 2900-fold purification. Overall yield was 35%. The different steps of purification are summarized in Table II. Identical results were obtained whether the enzymes were purified in the usual conditions, or in the presence of the various antiproteolytic substances detailed in Materials and Methods.

Polyacrylamide gradient slab gel electrophoresis of the undissociated enzymes

Fig. 3 shows the electrophoretic pattern of the pure F-type, M-type, L-type and red cell phosphofructokinases. The procedures used for the purification of the enzymes from muscle (M_n), granulocytes (L_n) and red cell ($L_nM_{n/2}$) have been previously described [1,9].

TABLE I

MAXIMUM IMMUNOPRECIPITATION OF PHOSPHOFRUCTOKINASE FROM PLATELETS BY THE DIFFERENT ANTISERA

Anti-M, anti-L and anti-F_2 refer to the absorbed antisera whose immunoglobulins have been purified [1,3]. Peaks 1—3 correspond to the chromatographic fractions eluted from the DEAE-cellulose column. The expected nature of the predominant tetrameric enzyme of each peak is indicated in parentheses.

	% of residual activity after precipitation by excess anti-phosphofructokinase antisera		
	Anti-M	Anti-L	Anti-F_2
Platelet crude extract	>90	26	14
Peak 1 (F_4)	80	70	<10
Peak 2 (F_3L)	>90	33	<10
Peak 3 (F_2L_2)	>90	14	21

TABLE II

HUMAN PLATELET PHOSPHOFRUCTOKINASE PURIFICATION

	Proteins (mg)	Activity (I.U.)	Specific (I.U./mg)	Purification (-fold)	Yield (%)
Cell extraction	48 210	1832	0.038	1	100
First DEAE-cellulose chromatography, batchwise	8 062	1290	0.16	4.2	70
$(NH_4)_2SO_4$ fractionation	3 750	1200	0.32	8.4	66
Second DEAE-cellulose chromatography					
Peak 1 (F_4)	667	400	0.6	15.8	
Peak 2 (F_3L)	714	500	0.7	18.4	63
Peak 3 (F_2L_2)	417	250	0.6	15.9	
Dextran Blue-Sepharose 4B chromatography					
F_4	2.1	295	142	3737	35
F-L hybrids	3.2	350	110	2895	

Muscle and platelet (peak 1) enzymes migrated as major forms slightly more anodic than ferritin, which is in agreement with the formulae M_4 and F_4; F_4 was slightly more anodic than M_4. Some minor protein bands could be observed in the upper part of the gel (corresponding to the lower acrylamide concentra-

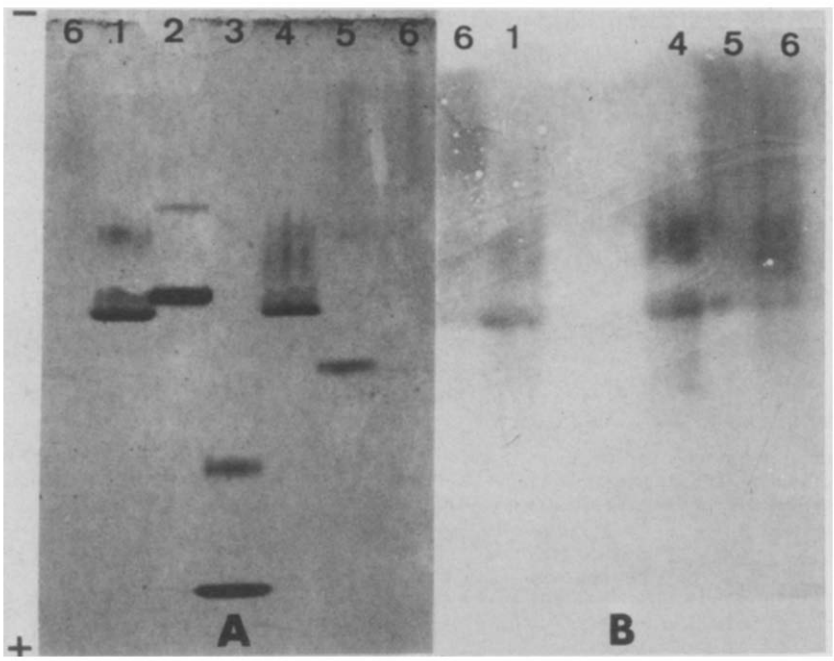

Fig. 3. Polyacrylamide gradient gel electrophoresis. Migration of 18 h at pH 8.9. (A) Staining with Coomassie blue. (B) Staining for phosphofructokinase activity. About 5 μg proteins were deposited on the gel for each sample. 1, pure platelet enzyme, peak 1; 2, ferritin; 3, bovine serum albumin; 4, pure muscle phosphofructokinase; 5, pure red cell phosphofructokinase; 6, pure granulocyte phosphofructokinase.

tions); some of them very likely corresponded to aggregates. In any case all the protein bands observed for the muscle and platelet enzymes exhibited some enzyme activity.

The patterns observed for L-type enzyme (and L-M hybrids from red cell) were very different: here the enzymes poorly entered the gel and yielded tailing protein and activity bands which probably corresponded to aggregates [10,11]. Moreover a protein band migrating in the more concentrated region of the gel and devoid of enzyme activity was observed, it was especially marked for red cell phosphofructokinase. According to its position in the gel we may suppose that this band might correspond to inactive trimeric forms, of L_3 formula.

In red cells, L_3 trimers could arise from the relative unstability of the L_3M hybrids which have been proven to be one of the major species of red cell phosphofructokinase [3,12].

SDS-polyacrylamide gradient slab gel electrophoresis

Figs. 4 and 5 show that the F_4 enzyme is, indeed, composed of a single (or

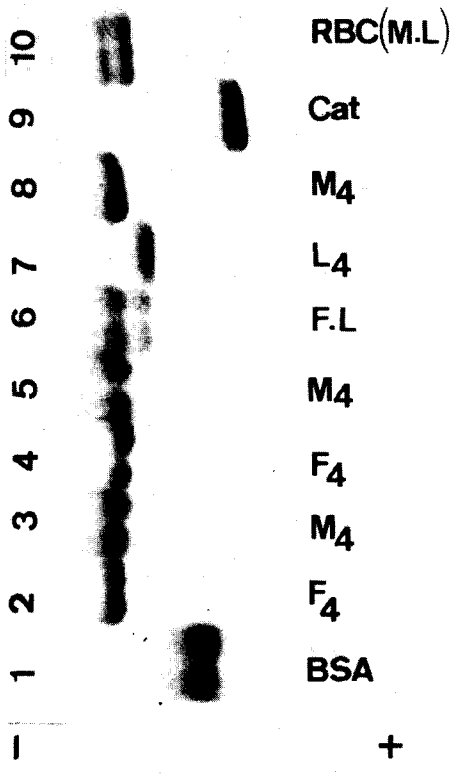

Fig. 4. SDS-polyacrylamide gradient gel electrophoresis. 5 μg protein/sample, staining with Coomassie blue. 1, bovine serum albumin; 2 and 4, platelet phosphofructokinase, peak 1; 3, 5 and 8, muscle phosphofructokinase; 6, platelet phosphofructokinase, peaks 2 + 3 (i.e. F-L hybrids); 7, L-type phosphofructokinase from granulocytes; 9, catalase; 10, red cell phosphofructokinase (composed of L and M subunits). The platelet enzymes analysed in this picture were purified in the usual conditions, without addition of the multiple antiproteolytic substances.

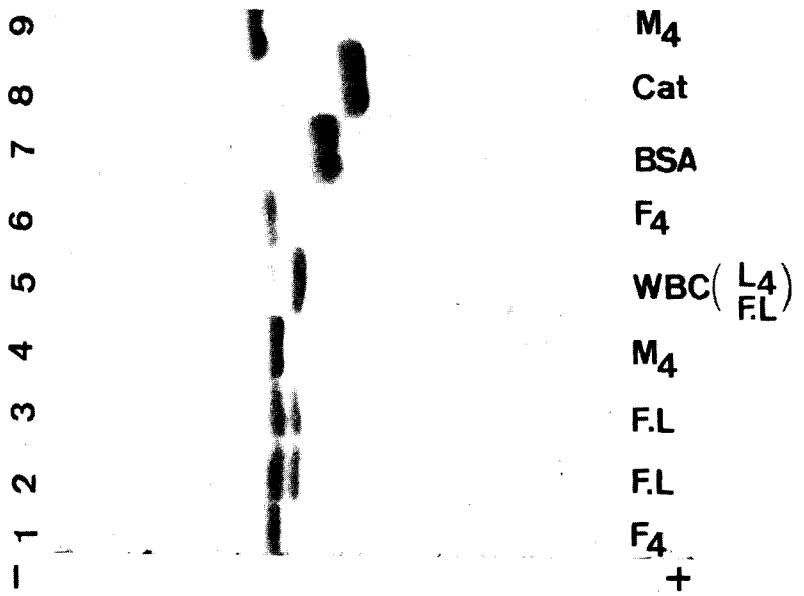

Fig. 5. SDS-polyacrylamide gradient gel electrophoresis. Comparison of platelet enzyme purified with or without addition of multiple antiproteolytic substances. 1, platelet enzyme, peak 1, +multiple antiproteolytic substances; 2, platelet enzyme, peaks 2 + 3, +multiple antiproteolytic substances; 3, platelet enzyme, peaks 2 + 3, with diisopropylphosphorofluoridate only; 4 and 9, muscle enzyme; 5, phosphofructokinase from leukemic granulocytes (composed of L_4 enzyme as major form and F-L hybrids as minor forms), 6, platelet enzyme, peak 1, +diisopropylphosphorofluoridate only; 7, bovine serum albumin; 8, catalase.

highly predominant) type of subunit with similar molecular weight to that of the M-type subunits (i.e. about 85 000). No contaminant was observed up to a protein load of 20 μg (it is not possible to apply more protein on this type of plate); such an overload, however, allowed us to detect a very slight band migrating as the L-type subunits and accounting for less than 10% of the total proteins. Phosphofructokinase considered as corresponding to the F_2L and F_2L_2 isozymes was, indeed, composed of two bands migrating similar to the F and L subunits, with the excepted ratio F/L. This phosphofructokinase preparation was as pure as the F_4 enzyme.

A similar molecular weight of the F subunits was obtained whether the purification was performed as usually, or in the presence of a mixture of antiproteolytic substances active on both serine proteases and cathepsins.

Immunological characterization

The immunoprecipitation tests confirmed the data represented in Table I: pure F_4 enzyme was significantly neutralized only by the anti-F antiserum whereas both anti-F and anti-L-type antisera were active on the F-L hybrids. Anti-M antiserum was, in the concentration range used, practically inefficient on both phosphofructokinase enzymes.

Fig. 6 represents double-immunodiffusion tests performed with all three specific antisera. With anti-F antiserum, precipitin lines were obtained with F_4

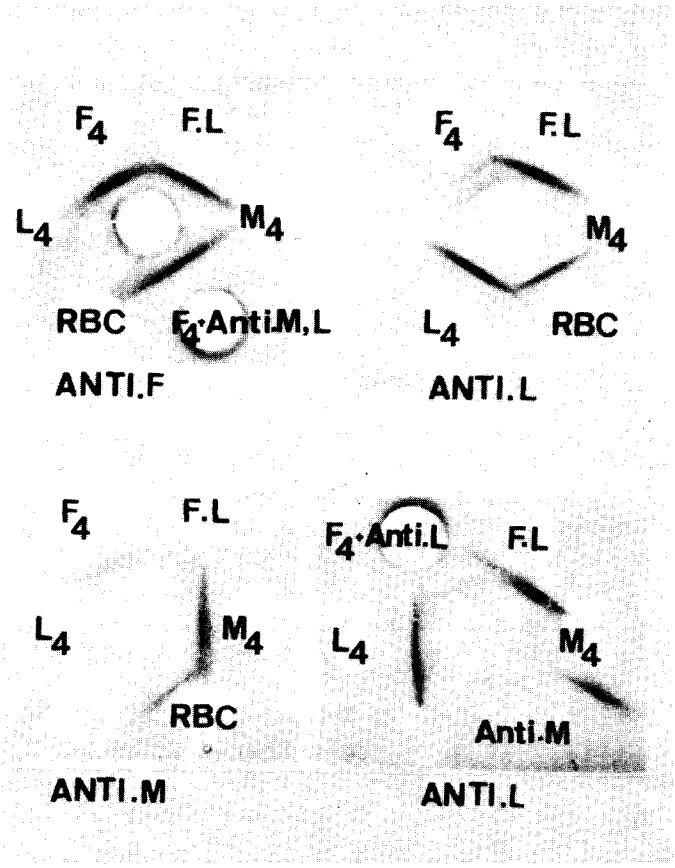

Fig. 6. Double-immunodiffusion analysis of the different forms of phosphofructokinase. Staining with amidoblack. The centre wells contained the antisera (i.e. purified immunoglobulins): 12.5 µl anti-F_2-type antiserum, 25 µl anti-L or anti-M type antisera. The outer wells were filled with: F_4 enzyme, (platelet phosphofructokinase, peak 1), 0.5 IU; F-L hybrids (platelet phosphofructokinase, peaks 2 + 3), 0.4 IU; M_4 enzyme from muscle, 0.5 IU; red blood cell (RBC) phosphofructokinase, 0.4 IU; L_4 enzyme from leukemic granulocytes, 0.3 IU. In the upper, left quarter of the picture one well was filled with 0.5 IU F_4 enzyme + 10 µl anti-M + 10 µl anti-L-type antisera. In the lower, right quarter one well was filled with 0.5 IU F_4 + 10 µl anti-L-type antiserum; another well was filled with 25 µl anti-M-type antiserum.

and F-L hybrids, but not with phosphofructokinase from red cells, muscle and granulocytes (in fact, a very slight line could be detected with the granulocyte enzyme, due to the presence in this preparation of a small amount of F-type subunits, as proved in Fig. 5). When mixed with both anti-M and anti-L-type antisera, the F_4 antigen was not precipitated and gave again an intense precipitin line with the anti-F-type antiserum.

With anti-L-type antiserum, intense precipitin lines were obtained with the F-L hybrids, red cell and granulocyte enzymes, but not with M-type phosphofructokinase. Two or three faint lines were obtained with F_4 enzyme; all of them could be specifically stained for phosphofructokinase activity (not shown), hence did not correspond to contaminants. In addition these lines exhibited a pattern of partial identity (i.e. a 'spur' phenomenon) with the

precipitin line corresponding to the F-L hybrids. When anti-L-type antiserum was added in the outer well containing the F_4 enzyme, a faint line was found again, with a pattern of partial identity with L_4 phosphofructokinase from granulocytes.

Anti-M-type antiserum gave an intense precipitin line with M_4 enzyme from muscle and a slight line with red cell phosphofructokinase (with a spur between them). Faint lines were also observed with F_4 and the F-L hybrids; they showed a pattern of partial identity with the line corresponding to M_4 phosphofructokinase.

Discussion

In a first paper [2] we reported, on the basis of a strong reactivity with anti-L-type antiserum, L-type subunits to be predominant in the platelets. In fact, as previously rectified [3], platelet phosphofructokinase is composed of various F-L hybrids and of some F_4 enzyme. This discrepancy with our earlier data is explained by the ability of anti-L-type antiserum to precipitate all the L-containing hybrids; this point was discussed into details in Ref. 2 and 3.

On a methodological point of view, we confirmed that the method of affinity chromatography on Dextran Blue-Sepharose 4B which we previously described 1[] enables the total purification of all the human phosphofructokinase forms.

Total purification and characterization of the F-type phosphofructokinase represents the first study of this type in man as well as in mammals. The existence of a third fundamental form in addition to the liver and muscle-type enzymes, however, has already been reported in rabbit and rat brain [13—15]. More recently it was shown that the minor isozyme of rat liver phosphofructokinase was immunologically not related to either muscle-type or liver-type enzyme [16,17]. Finally, a recent paper reports total purification of sheep kidney phosphofructokinase, but without any discussion of its isozymic nature and subunit composition [18]. In our laboratory we previously suspected the existence of this third basic form of human phosphofructokinase on electrophoretic and immunologic arguments [2]. We found that this form seemed to be present in cultured fibroblasts, brain, platelets and lymphocytes [2,3]. In pure brain enzyme, however, we detected three immunological specificity (M, L and F) but only two types of subunits distinguishable according to their molecular weights [1]. The reasons of this difficulty are now clear: F and M-type subunits have an identical molecular weight (i.e. about 85 000).

F_4 enzyme is nevertheless easily distinguished from M_4 phosphofructokinase. (i) the former is eluted from the Dextran Blue-Sepharose columns at high ionic concentration (200—250 mM KCl) while the latter is eluted at only 30 mM KCl. (ii) The immunoprecipitation tests with anti-M and anti-F antisera yield non-ambiguous results allowing to distinguish these forms. (iii) Genetically, phosphofructokinase activity of the patients deficient in muscle enzyme is normal in the platelets [19,20] and cultured fibroblasts (Mathieu, M., personal communication). As for the differences between the L and F-type enzyme, they are obvious: in addition to the difference of molecular weight, both enzymes have clearly different electrophoretic and chromatographic properties

and are also easily distinguished by their antigenicity [2,3]; by contrast, the elution properties from the Dextran Blue-Sepharose column are practically the same. Another unique property of L-type phosphofructokinase and L-containing hybrids is the easy aggregation in high molecular weight polymers [10,11], while the tetrameric forms are predominant for F-type and M-type enzyme (Fig. 3).

It is always difficult to affirm that molecular weight of a purified enyzme corresponds, in fact, to that of the native, recently synthesized enzyme: proteolysis phenomena may occur in vivo, with post-synthetic maturation or aging [21,22], and in vitro [22,23]. It is the reason why we verified molecular weight of the F subunits purified from a cell extract treated with various anti-proteolytic substances, especially with anti-cathepsins.

Although this type of approach does not definitely eliminates such a partial proteolysis in the course of purification it, at least, argues against this eventuality.

Although practically no relationship between M-type, L-type and F-type phosphofructokinase could be detected using immunoprecipitation tests, double immunodiffusion clearly showed that some immunological relations between them do exist. Anti-M-type antiserum, prepared against homogeneous M_4 phosphofructokinase and absorbed with hepatoma enzyme (containing L and F subunits), gives a slight precipitin line with F_4 and F-L hybrids, with a pattern of partial identity with muscle enzyme. Anti-L-type antiserum, also absorbed with F-type enzyme [1], recognized at least two types of antigen in the F_4 preparation. One of them corresponded to a line located close to the antigen well; it could be suppressed when anti-L-type antiserum was mixed with F_4. We may suppose that this antigen corresponded to a little amount of contaminant F_3L hybrid co-purified with F_4. The other antigenic determinants recognized by anti-L-type antiserum gave a slight precipitin line located close to the antiserum well, which may signify that the antiserum has a slight affinity for them. This line persisted after treatment of F_4 phosphofructokinase by the antiserum; it exhibited a pattern of partial identity with L_4. We assume that this latter type of antigenic structure could correspond to F_4 itself which could carry some common antigenic determinants with the L_4 enzyme. This slight antigenic relations between F_4 and the other basic forms of phosphofructokinase might reflect their common origin from an ancestral gene duplicated during evolution.

In conclusion, this paper reports on the first purification and characterization of F_4 phosphofructokinase. It is a tetrameric enzyme composed of subunits of 85 000 daltons. It exhibits slight immunologic relationship with the other phosphofructokinase forms. Kinetic properties of this enzyme are currently being studied in our laboratory.

Acknowledgements

We thank Ms. Fr. Carrouget for typing the manuscript and Robert Kernemp for his excellent technical assistance. This work was supported in part by a grant from INSERM (ATP 56.78.88).

References

1 Cottreau, D., Levin, M. and Kahn, A. (1979) Biochim. Biophys. Acta 568, 183—194
2 Kahn, A., Meienhofer, M.C., Cottreau, D., Lagrange, J.L. and Dreyfus, J.C. (1979) Hum. Genet. 48, 93—108
3 Meienhofer, M.C., Lagrange, J.L., Cottreau, D., Lenoir, G., Dreyfus, J.C. and Kahn, A. (1979) Blood 54, 389—400
4 Marie, J., Kahn, A. and Boivin, P. (1976) Biochim. Biophys. Acta 438, 393—406
5 Aoyagi, T., Morishima, H., Nishizawa, R., Kunimoto, S., Takeuchi, T., Umezawa, H. and Ikezawa, H. (1972) J. Antibiot. 25, 689—694
6 Aoyagi, T. and Umezawa, H. (1975) in Proteases and Biological Control (Reich, E., Rifkin, D.B. and Shaw, E., eds.), pp. 429—454, Cold Spring Harbor Laboratory, New York
7 Nevile, D.M. (1971) J. Biol. Chem. 246, 6328—6334
8 Laemli, U.K. (1970) Nature 227, 690—685
9 Lagrange, J.L., Marie, J., Cottreau, D., Fisher, S. and Kahn, A. (1979) Biochim. Biophys. Acta, in the press
10 Trujilo, J.L. and Deal, W.C. (1977) Biochemistry 16, 3098—3104
11 Wenzel, K.W., Kurganov, B.I., Zimmerman, G., Yakovlev, V.A., Schellenberger, W. and Hofmann, E. (1976) Eur. J. Biochem. 61, 181—190
12 Vora, S. and Piomelli, S. (1977) Blood 50, Suppl. 1, 87
13 Kurata, N., Matsushima, T. and Sugimura, T. (1972) Biochem. Biophys. Res. Commun. 48, 473—479
14 Tsai, M.Y. and Kemp, R.G. (1973) J. Biol. Chem. 248, 785—792
15 De Faria, J.B., El Dorry, H.A. and Bacila, M. (1978) Dev. Biol. 65, 547—552
16 Dunaway, G.A., Morris, H.P. and Weber, G. (1974) Cancer Res. 34, 2209—2216
17 Dunaway, G.A., Leung, G.L.Y., Cooper, M.D., Thrasher, J.R. and Wagle, S.R. (1978) Biochem. Biophys. Res. Commun. 80, 71—74
18 Mendicino, J., Leibach, F. and Reddy, S. (1978) Biochemistry 17, 4662—4669
19 Layzer, R.B., Rowland, L.P. and Ranney, H.M. (1967) Arch. Neurol. 17, 512—523
20 Kahn, A., Etiemble, J., Meienhofer, M.C. and Boivin, P. (1975) Clin. Chim. Acta 61, 415—419
21 Kahn, A., Bertrand, O., Cottreau, D., Boivin, P. and Dreyfus, J.C. (1977) Biochem. Biophys. Res. Commun. 77, 65—72
22 Kahn, A., Marie, J., Garreau, H. and Sprengers, E.D. (1978) Biochim. Biophys. Acta 523, 59—74
23 Chappel, A., Hoogenraad, N.J. and Holmes, R.S. (1978) Biochem. J. 175, 377—382