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REVERSIBLE MICROSOMAL BINDING OF HEPATIC ALDOLASE

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Fructose-1,6-bisphosphate aldolase (D-fructose-1,6-bisphosphate D-glyceraldehyde-3-phosphate lyase, EC 4.1.2.13) partitions between the microsomes and the cytosol when a rat liver homogenate is fractionated by differential centrifugation. Gel electrophoresis and immunodiffusion indicate that the one isozyme present in the liver of the young adult rat is found in both fractions. The association of the aldolase with membranes is differentially sensitive to a variety of metabolites and inorganic salts. In the absence of cellular salts, 1 mM fructose 1,6-bisphosphate or glucose 1,6-bisphosphate elutes 50% of the enzyme from the microsomes. About 9 mM P_i or citrate is necessary to produce the same effect. With other metabolites or inorganic salts higher concentrations are required. The fraction of total enzyme which partitions with the microsomes when a homogenate is submitted to high speed centrifugation, correlates inversely with the level of fructose 1,6-bisphosphate in the supernatant solution and this concentration is higher when the tissue concentration in the homogenate is greater. The K_m for fructose 1,6-bisphosphate of $3 \cdot 10^{-4}$ for aldolase bound to microsomes is decreased to $6 \cdot 10^{-6}$ M when the enyme is dissociated from the membranes with salt. These observations appear relevant to the ongoing discussion regarding the physiological relevance of the subcellular localization of glycolytic enzymes.

Introduction

Green et al. [1] first suggested that fructose-1,6-bisphosphate aldolase was localized intracellularly when substantial amounts of the enzyme were found in the particulate fraction obtained from bovine erythrocytes or *Saccharomyces cerevisia*. Subsequently, other associations between fructose-1,6-bisphosphate aldolase and specific subcellular structures such as the microsomal membranes and components of muscle were reported [2–9]. For example, histochemical techniques confirmed the localization of fructose-1,6-bisphosphate aldolase in the I-band region of rabbit muscle [8,9]. Similarly, ultrastructural immunocytochemical techniques suggested that fructose-1,6-bisphosphate aldolase is associated with the endoplasmic reticulum in the hepatocyte of the rat [10]. It has also been reported that the enzyme is localized in the hepatic nuclei of the rat [11,12] and on the inner surface of the membranes in human erythrocytes [13].

Various authors [1,3,4,6,10,11,14] have proposed that the binding between particular subcellular components and fructose-1,6-bisphosphate aldolase is specific because of the greater sensitivity of the association to molecules which are specific ligands (i.e., substrates, products and effectors) of the enzyme than to other glycolytic intermediates, inorganic salts or pH. Changes in kinetic parameters when an enzyme is associated or dissociated are cited as support for the postulated specificity of the

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interaction. Fructose-1,6-bisphosphate aldolases from brain and muscle have been reported to exhibit different kinetics when associated with subcellular or artificial structures as compared with the situation for enzyme in the free state [4,15].

Data in the present report show with respect to fructose-1,6-bisphosphate aldolase in rat liver, that the enzyme isolated from the microsomal fraction is the same isozyme as is found in the high-speed supernatant solution, that the V remains the same but the K_m is about 50-fold greater when the enzyme becomes associated with the microsomes, that fructose 1,6-bisphosphate and glucose 1,6-bisphosphate are 10-fold more efficient in dissociating the aldolase from the microsomes than is any other metabolite tested, that the percentage of aldolase found in association with the microsomes correlates inversely with the concentration of fructose 1,6-bisphosphate in the tissue homogenate at the time when the microsomes are being sedimented and that the concentration of fructose 1,6-bisphosphate can rise significantly during homogenization and fractionation if the tissue represents more than 10% of the homogenizing volume.

Materials and Methods

Fractionation of rat liver and brain homogenates. Rat liver as 10% (w/v) homogenates in 250 mM sucrose/1 mM EDTA (pH 6.0) (Solution 1) was fractionated into microsomal and high-speed supernatant fractions as described by Foemmel et al. [10]. The aldolase found in the high-speed supernatant solution was designated free aldolase. The enzyme in the microsomal pellet, in 22% (w/v) suspension in Solution 1, was termed bound aldolase. To obtain the free form of the bound enzyme, the microsomal pellet was suspended in either 250 mM sucrose/150 mM NaCl/1 mM EDTA (pH 7.6) (Solution 2), or 20 mM Tris-HCl/150 mM NaCl/1 mM EDTA (pH 7.6) (Solution 3) for 30 min at 4°C, after which the microsomes were sedimented at high speed [10], leaving the aldolase in the supernatant solution. To obtain preparations of total aldolase, the homogenizing medium was made to 150 mM in NaCl and the enzyme was recovered in the high-speed supernatant solution. In a representative experiment, 7 units enzyme/g tissue were found in the homogenate, of which 67% was associated with the microsomal fraction and 41% was found in the high-speed supernatant solution. Rat brain aldolase used as a standard for separation of isozymes by electrophoresis was a total aldolase preparation obtained by the same procedure.

Dissociation of fructose-1,6-bisphosphate aldolase from microsomes affected by inorganic salts and metabolites. Microsomal fractions were washed with 1 vol. (1 ml/g original weight of liver) and then resuspended at 4.5 ml/g liver in Solution 1 before diluting 18-fold in Solution 1 containing concentrations of inorganic salts or metabolites designed to yield the desired final concentration. The diluted mixtures were allowed to stand for 30 min at 2°C with occasional shaking. The microsomes were then removed by centrifugation at $100\,000 \times g$ for 1 h at 4°C, and the supernatant solution was assayed for aldolase activity. If residual activity on the microsomes was to be determined, the pellet was washed with and resuspended in Solution 1.

Reconstitution of aldolase-containing membranes. Microsomal pellets were suspended in Solution 3, allowed to stand for 1 h at 4°C and centrifuged at $100\,000 \times g$ to remove all detectable aldolase from the membranes. The stripped microsomes were suspended (22%, w/v) in Solution 1. Aldolase, in an equivalent quantity to that removed, was added to the suspension of stripped microsomes and the mixture, after standing for 30 min at 4°C, was dialyzed against 100 vol. Solution 1 for 4 h at 4°C. After dialysis, the microsomes were recovered by centrifugation at $100\,000 \times g$ for 1 h at 4°C and the aldolase activity was determined in the supernatant solution and resuspended pellet. About 80% of the enzyme had become reassociated with the microsomes by the procedure.

Purification of fructose-1,6-bisphosphate aldolase. Free, bound and total rat liver aldolase were purified as described by Foemmel et al. [10] with the following modifications: the crude enzyme extracts were dialyzed against 20 mM Tris-HCl/1 mM EDTA (pH 7.6) prior to fractionated on two successive phosphocellulose columns. The eluant from the second column of each preparation yielded one protein band coincident with a band stained for activity in polyacrylamide gel electrophoresis.

The final recovery of total aldolase was 32% with a

purification of 53-fold based upon the specific activity of 0.063 unit/mg protein in the crude homogenate assayed at pH 7.6 and 37°C. Protein was determined by the method of Lowry et al. [16]. For each gram of liver (wet weight) about 9.5 units of total aldolase were obtained in the homogenate. 10 and 21%, respectively, of bound and free aldolase, at comparable specific activities, were recovered based upon the activities in the microsomal and high-speed supernatant fractions. Specific activities of 1.49 units/mg at 25 [17], 1.3 units/mg at 30 [18] and 1.28 units/mg at 30°C [19], for aldolase from rat liver have been reported previously.

Assay for aldolase activity. Fructose-1,6-bisphosphate aldolase activity was determined spectrophotometrically at 37°C as described by Blostein and Rutter [20] with the following modifications: the conditions for measuring fructose-1,6-bisphosphate aldolase activity in the kinetic studies of free and bound aldolase consisted of 0.12 mM NADH/5.8 units α-glycerophosphate dehydrogenase/44 units triosephosphate isomerase/5.0 mM Tris-HCl (pH 6.0)/ approx. 0.008 units aldolase (suspended in Solution 1)/0.012–0.2 mM fructose 1,6-bisphosphate, in a final volume of 3 ml. The reaction conditions used for all other studies consisted of 0.12 mM NADH/ 0.03 units α-glycerophosphate dehydrogenase/2.0 units triosephosphate isomerase/0.15 mM fructose 1,6-bisphosphate/54 mM Tris-HCl (pH 7.6)/approx. 0.004 units aldolase, in a final volume of 3 ml. Initial velocities were analyzed with a Hewlett-Packard 2000C digital computer with a BASIC program for fitting velocities to a hyperbolic function by an iterative least-squares method with symmetrical variance. Confidence intervals were calculated from the standard error by the Student test for kinetic values.

Assays for fructose-1,6-bisphosphate and combined triosephosphates. Fructose 1,6-bisphosphate was determined spectrophotometrically at 37°C with the combined enzyme system of Blostein and Rutter [20] using commerical preparations of aldolase, triosephosphate isomerase and α-glycerophosphate dehydrogenase. Glyceraldehyde 3-phosphate and dehydroxyacetone phosphate were assayed together using the same enzyme system minus aldolase.

Discontinuous polyacrylamide gel electrophoresis. Three replicate samples of an aldolase preparation were electrophoresed using a 5.5% polyacrylamide separating gel, pH 8.9, overlayed with a 2.5% stacking gel, pH 6.7, as described by Davis [21]. The protein was stained with Coomassie blue R-250 dye on one replicate. Fructose-1,6-bisphosphate aldolase activity was stained by a modification of the procedure utilized by Penhoet et al. [22] on another replicate. The gel was kept light free in 5 mM Na_2HAsO_4/20.8 mM fructose 1,6-bisphosphate/1.9 mM NAD^+/1.0 mM nitroblue tetrazolium chloride/0.2 mM phenazine methosulfate/51.4 mM pyrophosphate buffer (pH 7.6)/0.82 units/ml glyceraldehyde-3-phosphate dehydrogenase and incubated for 1.5 h at 37°C before scanning for absorbance at 550 nm. The third replicate was submitted to the enzyme assay without substrate as a control.

Preparation and use of antisera to fructose-1,6-bisphosphate aldolase. New Zealand white rabbits received three injections at intervals of 7 days (0.1 ml in each foot pad and 0.1 ml subscapularly and subcutaneously). Each injection contained 500 μg purified aldolase in 150 mM NaCl emulsified with an equal volume of Freund's complete adjuvant. The immune sera were collected 7 days after the last injection. Preimmune serum was obtained from each rabbit prior to innoculation.

Immunodiffusion was performed by the method of Kabat and Mayer [23]. Precipitation of aldolase was carried out in 10 mM phosphate buffer (pH 7.0)/150 mM NaCl for 48 h at 4°C using 12 μg bound aldolase or 9.4 μg/ml free aldolase and increasing amounts of the appropriate antiserum (previously dialyzed against the phosphate-saline mixture) followed by sedimentation at 10000 × *g* for 15 min and assay of the supernatant solution [10].

Materials. Adult rats of the CFN strain (Carworth Farms, Rockland, NY) maintained as a randomly inbred colony in this laboratory were used in these studies. Rabbit muscle fructose-1,6-bisphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase, α-glycerophosphate dehydrogenase and triose isomerase were purchased from Sigma Chemical Co. (St. Louis, MO). Reagents were purchased as follows: chemicals for electrophoresis from Matheson, Coleman and Bell (Lyndhurst, NJ); EDTA from Eastman (Rochester, NY); phosphocellulose and ultrapure sucrose from Schwartz-Mann (Spring Valley, NY); fructose 1,6-bisphosphate, NAD^+,

NADH and phenazine methosulphate from Sigma Chemical (St. Louis, MO), and nitroblue tetrazolium chloride from Aldrich Chemical (Milwaukee, WI).

Results

Identity of the microsomal and soluble fructose-1,6-bisphosphate aldolase

Samples of aldolase isolated from the microsomal and from the high-speed supernatant fractions of five separate rat liver homogenates made in Solution 1 were compared to determine whether the fructose-1,6-bisphosphate aldolase found in these two compartments represented the same or different isozymes. Fig. 1 shows the patterns obtained consistently on polyacrylamide gel electrophoresis of fructose-1,6-bisphosphate aldolase obtained from the various preparations of liver and from rat brain (three different preparations) run under identical conditions. Whereas five isozymes of aldolase were always obtained with the preparation from brain, only one isozyme was ever observed for liver. The enzyme from the microsomes (after dissociation from the membranes in Solution 2 or 3 and centrifugation), those from the high-speed supernatant solution and the entire homogenate, yielded the same electro-

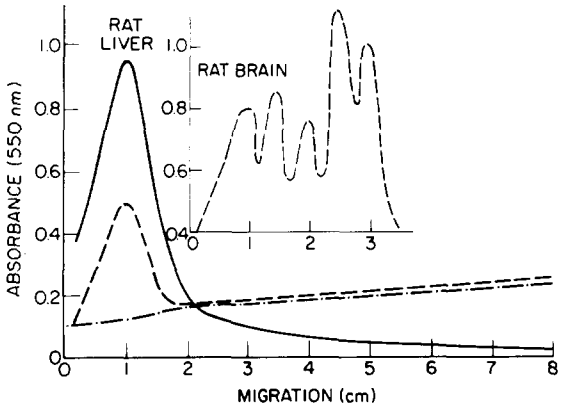

Fig. 1. Densitometer scan of polyacrylamide gel electrophoresis patterns of fructose-1,6-bisphosphate aldolase from rat brain (insert) and from rat liver microsomes, high-speed supernatant solution and crude homogenate. The patterns from the liver fractions were essentially indistinguishable and are represented by the same curve. Enzymatic activity was measured by absorbance at 550 nm with (------) and without (· – · – ·) fructose 1,6-bisphosphate. Protein was also measured at 550 nm (———).

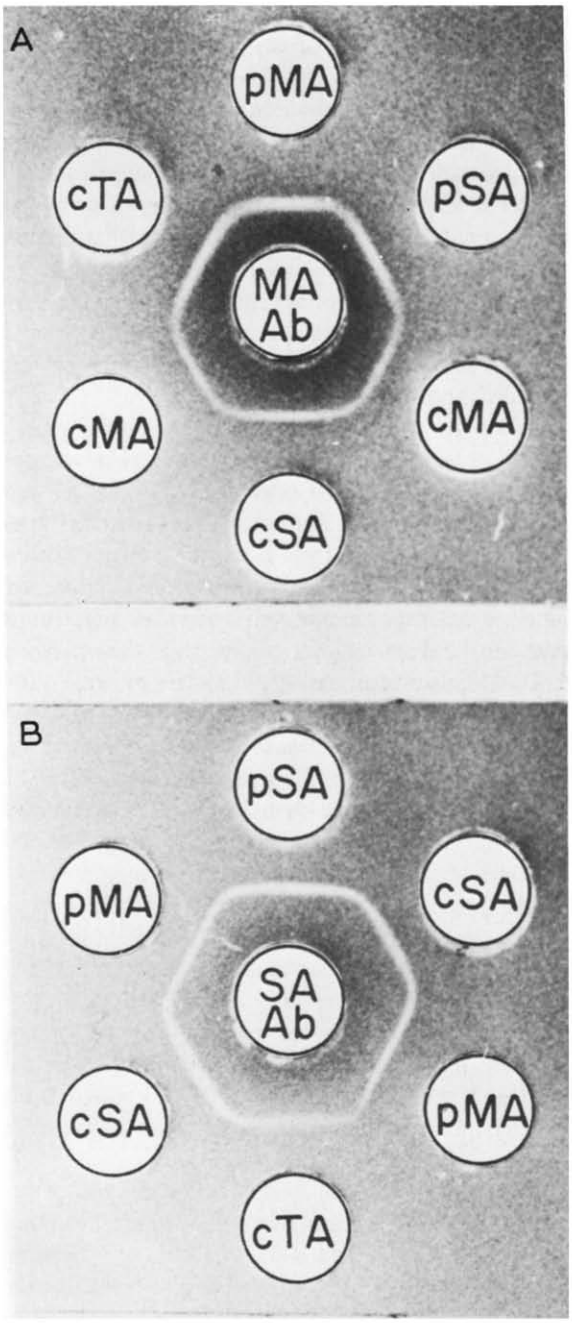

Fig. 2. Immunodiffusion of antimicrosomal (A) and antisoluble (B) aldolase against crude and pure forms of bound, free and total aldolase. pMA, pure microsomal aldolase; cMA, crude microsomal aldolase; pSA, pure soluble aldolase; cSA, crude soluble aldolase; cTA, crude total aldolase; $^{MA}_{Ab}$, antimicrosomal serum and $^{SA}_{Ab}$, antisoluble serum.

phoretic pattern every time. Immunodiffusion also indicated the identity of the enzyme obtained from these three preparations in five separate experiments. As shown in Fig. 2, sharp continuous precipitin bands with no spurs were obtained when antiserum against either purified free or bound enzyme was tested against free, bound and total enzyme. Preimmune serum used as a control, produced no precipitin bands. About 70% of the free aldolase was precipitated from solution when antiserum to the bound enzyme was added and about 80% of the bound enzyme was precipitated when antiserum to the free enzyme was used.

Effect of metabolites and inorganic salts on the binding of aldolase to microsomes

Fig. 3 shows the ability of various metabolites and inorganic salts to dissociate fructose-1,6-bisphosphate aldolase from washed microsomes. Glucose 1,6-bisphosphate was nearly as effective as fructose 1,6-bisphosphate in eluting the enzyme. A 1 mM solution eluted 50% of the enzyme. The monophosphates of these sugars were much less effective. Mono-, di- and tricarboxylic acids appeared to be increasingly effective in that order, as were Cl^-, SO_4^{2-} and P_i. Half of the enzyme was dissociated by 9 mM P_i. An equimolar mixture of D and L-glyceraldehyde 3-phosphate was much less effective than fructose 1,6-bisphosphate. If the elution is assumed to be a function of only the metabolically active isomer, the ability of the triosephosphate to dissociate the enzyme from the membranes is still much less than is the case for fructose 1,6-bisphosphate. Dihydroxyacetone-phosphate was also much less effective than fructose 1,6-bisphosphate. No enzyme was eluted in 11.8 mM dihydroxyacetone-phosphate. In order to verify that the enzyme was indeed eluted and not inactivated, the activity in the supernatant solution was determined after dialysis. In the case of elution with fructose 1,6-bisphosphate, 88% of the activity was found in the supernatant solution when 90% of the activity was dissociated from the microsomes. Similarly, 70 and 80%, respectively, were obtained when glyceraldehyde 3-phosphate was used to dissociate the enzyme from the membranes.

Relation of the percentage of aldolase found in the microsomal fraction to the concentration of fructose 1,6-bisphosphate and combined triosephosphates observed in the high-speed supernatant solution

Fig. 4 compares the concentrations of fructose 1,6-bisphosphate and combined triosephosphates

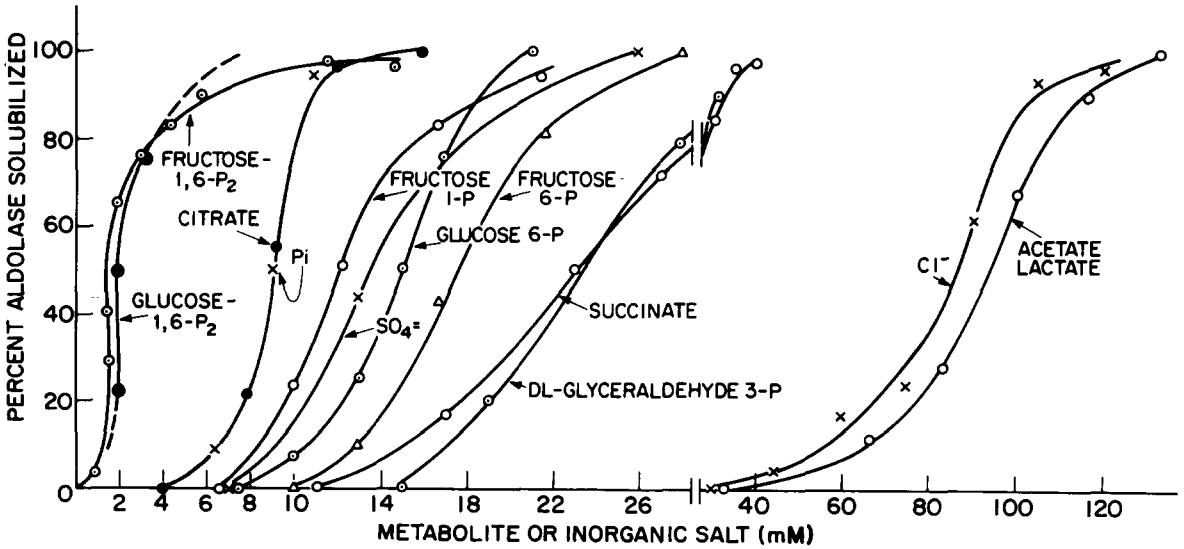

Fig. 3. Solubilization of microsomal fructose-1,6-bisphosphate aldolase by various metabolites and inorganic salts. Microsomes in Solution 1 (4.5 ml/g liver) were assayed for aldolase and were diluted 18-fold in Solution 1 containing the ion or metabolite being tested. After 30 min at 20°C, the microsomes were removed by centrifugation at 100 000 × *g* for 1h at 4°C and the supernatant solutions were assayed for the enzyme. The percent of enzyme solubilized was determined by assaying the supernatant solution.

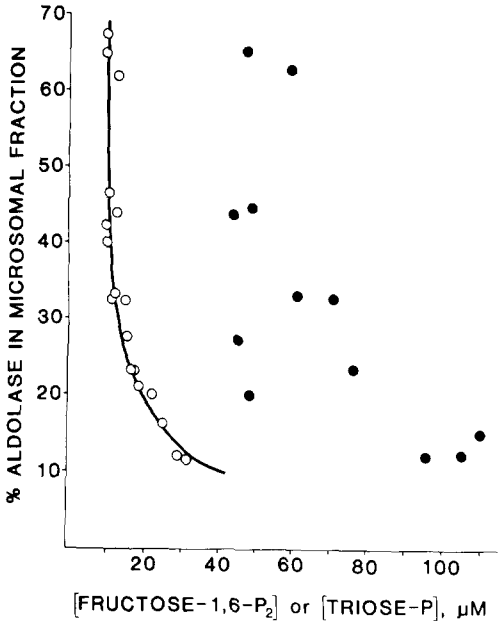

Fig. 4. Relation of concentration of fructose 1,6-bisphosphate (○) and combined glyceraldehyde-3-phosphate and dihydroxyacetone-phosphate (●) in high-speed supernatant solution to percentage of aldolase in microsomal fraction.

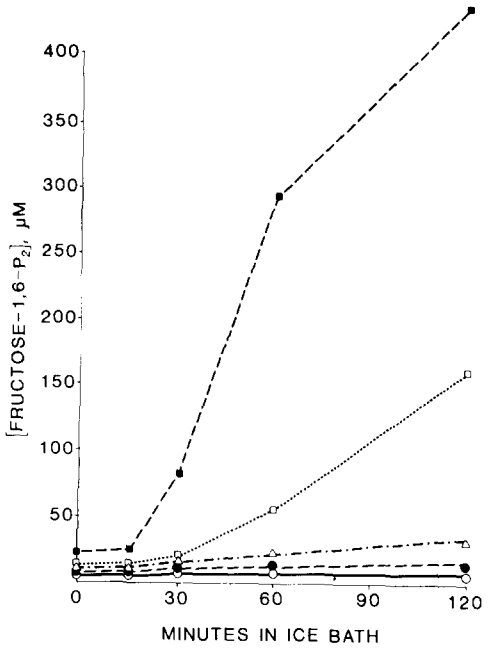

Fig. 5. Accumulation of fructose 1,6-bisphosphate in homogenate during isolation procedure. Homogenates were prepared at different ratios of weight of tissue to volume of Solution 1 (%), kept in an ice bath and assayed spectrophotometrically at different times for the concentration of fructose 1,6-bisphosphate using commercial aldolase, triose-phosphate dehydrogenase and α-glycerophosphate dehydrogenase. The tissue concentration of fructose 1,6-bisphosphate determined in these experiments can be obtained by multiplying the value at zero time by the dilution of tissue in the homogenate. The percentages represent the tissue concentration (w/v) in the homogenates. ■ – – – ■, 50%; □ - - - - - - □, 25%; △ · – · – · △, 13%; ● – – – ●, 10%; ○———○, 5%.

present in the high-speed supernatant solution with the percentage of aldolase found in the microsomal fraction from a series of homogenates. In contrast to the data for triosephosphate, a striking correlation was observed between the concentration of fructose 1,6-bisphosphate and the percentage of enzyme bound. The binding was clearly more sensitive to the presence of fructose-1,6-bisphosphate than to the combined triosephosphates.

Since a significant and variable time was expended in the isolation procedure, and the binding of aldolase to the microsomes was found to be sensitive to small changes in the concentration of fructose 1,6-bisphosphate, an experiment was done to evaluate changes which might occur in the concentration of fructose 1,6-bisphosphate during the isolation technique as applied to different dilutions of tissue in the homogenizing medium. Aliquots of homogenates made with different tissue concentrations were allowed to sit in an ice bath for different lengths of time prior to deproteinization by addition of 0.3 vol. of 5% $HClO_4$ followed by centrifugation at 8 000 × g for 10 min at 4°C. The concentrations of fructose 1,6-bisphosphate determined in the supernatant solutions are shown in Fig. 5. The values obtained at zero time are higher than those obtained by Greenbaum et al. [24] who took care to freeze the tissue quickly. Significant increases in the concentration of fructose 1,6-bisphosphate occurred in all cases with a tissue concentration above 10%. The rate at which the level of fructose 1,6-bisphoshate increased was greater when the concentration of tissue in the homogenate was higher.

K_m and V of bound and free fructose-1,6-bisphosphate aldolase

Although the V is not affected, the bound and free

forms of the enzyme have different values of K_m for fructose 1,6-bisphosphate. A K_m of $6 \cdot 10^{-6} \pm 0.3$ M for fructose 1,6-bisphosphate was exhibited by the purified aldolase. When the enzyme was adsorbed to previously stripped microsomes, the K_m for the substrate changed to $3 \cdot 10^{-4} \pm 0.15$ M. A value of $2.9 \cdot 10^{-4} \pm 0.15$ M was obtained when the crude enzyme associated with the membranes as isolated in the microsomal fractions was assayed. Addition of 150 mM NaCl to the same assay mixture, a procedure which caused the enzyme to dissociate from the membranes, resulted in the determination of a K_m of $5.3 \cdot 10^{-6} \pm 0.3$ M for the substrate. Addition of 150 mM NaCl to the assay mixture when purified enzyme was assayed with or without microsomes produced a K_m of $6 \cdot 10^{-6} \pm 0.3$ M for fructose 1,6-bisphosphate. The V remained unchanged by any of these manipulations. These analyses were repeated at least three times with five different preparations of enzyme and microsomes.

Discussion

The results from gel electrophoresis (Fig. 1) and immunodiffusion (Fig. 2) indicate that the aldolase associated with the microsomal fraction is the same protein as the aldolase present in the high-speed supernatant fraction of tissue homogenates. Direct demonstration of both the dissociation of microsomal aldolase by a variety of agents (Fig. 3) and the reassociation of aldolase with stripped microsomes provide further support for this view. Furthermore, the striking contrast in kinetic properties between the microsomal and free aldolase was found to be the same whether the bound enzyme was isolated from the homogenate on the microsomes or was formed by reassociation of the free aldolase with stripped microsomes.

Previously, Gracey et al. [17] reported a single electrophoretic activity band on cellulose acetate for rat liver aldolase, but Lebherz and Rutter [25] reported three activity bands. Results obtained in the present study indicate that the presence of isozymes at comparable levels at least is not likely. Polyacrylamide gel electrophoresis of the crude and purified aldolase showed a single protein band coincident with aldolase activity. Moreover, immunodiffusion of antiserum to the purified aldolase provided no evidence for the existence of aldolase isozymes in the crude extract of young adult rat liver. Weber et al. [26] have also reported the presence of only one isozyme in normal hepatocytes as assayed by ultrastructural immunocytochemistry.

The possibility that the observed association of hepatic aldolase with microsomes may have physiological implications was examined in terms of the relative abilities of different metabolites to dissociate the enzyme from the membranes. With isolated and washed microsomes, the solubilization of the associated aldolase was most sensitive to fructose 1,6-bisphosphate and glucose 1,6-bisphosphate in the range of 1–3 mM (Fig. 3). This concentration was at least an order of magnitude less than that required for glyceraldehyde-phosphate, dihydroxyacetone-phosphate or any of the other hexose monophosphates to dissociate the enzyme from the membrane. P_i and citrate were somewhat more effective than the hexosemonophosphates in this regard. Moreover, from the data in Fig. 4 it appears that slight changes in the concentration of fructose 1,6-bisphosphate in the presence of cellular salts and metabolites could be sufficient to exert a large effect on the dissociation of the enzyme. Since the intracellular concentration of fructose 1,6-bisphosphate is reported to be in the range of greatest sensitivity (Fig. 4), assuming homogeneous distribution within the cell [27,28], the association of fructose-1,6-bisphosphate aldolase with the membranes of the endoplasmic reticulum could conceivably be subject to regulation by slight changes in the physiological level of the substrate for this enzyme.

The results obtained in this study of the reversible association of liver aldolase with microsomal membrane fragments are reminiscent of those reported by Arnold and Pette [3,4] who studied the association of various purified muscle proteins with muscle aldolase. These investigators found that both the K_m for fructose 1,6-bisphosphate and the V were altered when the A_4 isozyme of muscle aldolase was bound to F-actin. The activity of fructose 1,6-bisphosphate isolated from the tuna was also increased 3-fold when bound to triosephosphate dehydrogenase from the same tissue [29]. Other studies of the association of glycolytic enzymes with various subcellular structures have yielded data interpreted as indicating that many of these enzymes are associated with specific

subcellular structures. In the case of hexokinase, the association appears to be specifically modulated by ligands [30,31] with the bound and free forms yielding different kinetic parameters [32–34]. Histochemical techniques have confirmed the localization of lactic acid dehydrogenase, triosephosphate isomerase, enolase, triosephosphate isomerase and glucose-6-phosphate isomerase as well as fructose-1,6-bisphosphate aldolase, in the I-band region of rabbit muscle [8,9,35]. Hexokinase was found to be associated in mitochondria [31,34], and glyceraldehyde-3-phosphate dehydrogenase along with fructose-1,6-bisphosphate aldolase were localized on the inner surface of the human erythrocyte membrane [13,36].

The physiological relevance of the reversible association between various glycolytic enzymes and subcellular structures has been questioned by Arion and Lange [37]. These workers, noting that most microsomal and soluble fractions have been derived from homogenates made after addition of 9 vol. (ml/g) of low-salt sucrose solution, prepared soluble fractions of rat liver by homogenization in 0.5 vol. 250 mM sucrose and ultracentrifugation. Supernatant solutions prepared under their conditions contained 97% of the aldolase activity originally observed in the homogenate. These workers concluded that any aldolase found in the microsomal fraction was a technical artifact resulting from the dilution of cellular salts. The data in Table I confirm the results obtained by Arion and Lange [37]. However, as shown in Fig. 5, there is in the more concentrated homogenates a rapid accumulation of fructose 1,6-bisphosphate which could account for the quantitative solubilization of the enzyme under these preparative conditions. A comparison of the data in Figs. 4 and 5 indicates that no more than 15% of the aldolase will be found associated with the microsomes in a homogenate having a tissue concentration of 13% or greater if the isolation takes as much as 1 h. In view of these rapid changes, experimental conditions which freeze the metabolic state of the tissue prior to microsomal separation must be utilized when investigating the in situ alteration of fructose 1,6-bisphosphate concentrations with respect to aldolase localization. A 10-fold dilution of the tissue homogenization is the most convenient although not the most effective method of minimizing metabolic change. Even so, sedimentation of microsomes should be accomplished as quickly as possible after excision of the tissue.

TABLE I

EFFECT OF TISSUE CONCENTRATION IN HOMOGENATE ON PERCENTAGE OF BOUND ALDOLASE

10% tissue concentration = 10 g tissue (wet weight) plus 90 ml homogenizing buffer (Solution 1). 100% activity in microsomal fraction = activity in soluble fraction plus activity in microsomal fraction.

% Tissue concentration	% Activity in microsomal fraction
5	69
10	67
11	45
12,5	32
15	16
20	12
25	5

The data reported in this paper are in accord with the hypothesis but do not pove that the reversible binding of aldolase to cellular membranes has physiological relevance. Support for this concept is being sought by experiments in which the level of fructose 1,6-bisphosphate in the tissue is altered by administration of suitable drugs to rats or by dietary manipulation and the level is correlated with the fraction of total aldolase associated with the microsomes. The association of the enzyme with the membranes in situ is determined by measuring the aldolase cross-linked to the microsomes by perfusing the liver with a dilute solution of glutaraldehyde prior to homogenization. This cross-linking technique has been used to investigate the localization of aldolase in human erythrocytes [38].

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