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# FRUCTOSE 1,6-DIPHOSPHATASE OF MOUSE EHRLICH ASCITES TUMOR AND ITS COMPARISON WITH THE ENZYMES OF LIVER AND SKELETAL MUSCLE OF THE MOUSE

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SUMMARY

1 The enzyme fructose 1,6-diphosphatase (D-fructose-1,6-diphosphate 1-phosphohydrolase, EC 3.1 3 11) has been identified in extracts of mouse Ehrlich ascites carcinoma The tumor cells have higher activity than mouse skeletal muscle. Fructose diphosphatase was partially purified from mouse Ehrlich ascites tumor, liver and leg muscle.

2. The partially purified Ehrlich ascites tumor fructose diphosphatase is inhibited by substrate concentrations greater than 0 05 mM. At non-inhibitory concentrations of substrate, a $K_m$ of 8 1 $10^{-6}$ M was measured. At 0 1 mM Fru-1,6-$P_2$, the optimum pH is 7.4 and at this pH maximum activity requires $Mg^{2+}$ (or $Mn^{2+}$) and EDTA. These properties are similar to those of fructose diphosphatase from rat liver.

3 Like fructose diphosphatase identified in other tissues, the tumor enzyme is inhibited by AMP At pH 7.4 and with 0.1 mM Fru-1,6-$P_2$, 50% inhibition requires 1.3 $\mu$M AMP The corresponding values for the enzymes from mouse liver and leg muscle are 130 and 2 1 $\mu$M, respectively Therefore, regarding the sensitivity to inhibition by AMP, the tumor enzyme resembles the muscle enzyme rather than the liver enzyme.

4. With tumor extracts, but not with liver or muscle extracts, a lag period of about 10 min was observed in the assay measurement of fructose diphosphatase. Evidence is presented which suggests that, during the lag period, the fructose diphosphatase is inhibited by AMP present in the extracts

## INTRODUCTION

We have previously reported that in Ehrlich ascites tumor cells, [6-$^{14}$C]glucose can be oxidized to $^{14}CO_2$ through resynthesis of Glc-6-*P* from pentose cycle-generated glyceraldehyde 3-phosphate[1]. The oxidation requires the conversion of Fru-1,6-$P_2$ to Fru-6-*P* and, in the course of investigating the mechanism of this conversion, we have found significant fructose 1,6-diphosphatase (D-fructose-1,6-diphosphate 1-phospho

hydrolase, EC 3 1.3 11) activity in extracts of Ehrlich ascites tumor cells[1]. This finding was rather surprising since the tumor had been said to lack the enzyme[2] Although rat liver has a very active fructose diphosphatase[3,4], WEBER AND MORRIS[5] showed that the enzyme was markedly decreased or absent in rapidly growing rat hepatomas

The present report deals with the partial purification and characterization of fructose diphosphatase from mouse Ehrlich ascites tumor cells. In addition, fructose diphosphatase was purified from liver and leg muscle of mouse and their properties were compared with those of the tumor enzyme These studies have revealed that the tumor enzyme resembles the muscle enzyme in being extremely sensitive to inhibition by AMP.

## MATERIALS AND METHODS

### *Tumor cells*

A hyperdiploid Ehrlich ascites carcinoma strain carried in mice of the *dd*-strain was used The tumor cells were harvested from the peritoneal cavity of the mice 8–11 days after inoculation and washed twice in cold physiological saline to remove erythrocytes and other contaminants

### *Preparation of tumor extracts*

The tumor cells were washed once in isotonic (0 154 M) KCl containing 1 mM EDTA (neutralized), resuspended in the same medium and centrifuged at 1000 × *g* for 5 min. To these packed cells, 2 vol of cold distilled water were added and the mixture was homogenized at 0° in a Teflon pestle homogenizer for 5 min with several resting intervals. The homogenate was made isotonic by the addition of 0.308 M KCl containing 2 mM EDTA and centrifuged at 105 000 × *g* for 30 min at 2° The supernatant (extract) was used for enzyme activity assays

### *Preparation of liver or muscle extracts*

Normal, well-fed mice, weighing 35–45 g, were killed by decapitation The livers or leg muscles were quickly removed, rinsed with physiological saline, blotted on filter paper and cut into small pieces. The livers were then homogenized at 0° in a Teflon pestle homogenizer with 9 vol. of 0 154 M KCl containing 1 mM EDTA. For the preparation of muscle homogenates, a Waring blender was used in place of the Teflon homogenizer The homogenates were centrifuged at 105 000 × *g* for 30 min at 2° and the supernatants (extracts) were used for enzyme activity assays

### *Partial purification of fructose diphosphatase*

Unless otherwise specified, all operations were carried out at 0–4° and all centrifugations were at 15 000 × *g* for 10 min.

An aliquot of tumor extract was dialyzed against approx 500 vol. of 1 mM EDTA, pH 7 0. This treatment resulted in an almost complete inactivation of phosphofructokinase (EC 2.7.1 11). To the dialyzed extract, 0.1 vol. of 1% protamine sulfate was added and the precipitate formed was removed by centrifugation The supernatant was then fractionated with increasing concentrations of $(NH_4)_2SO_4$ and the fraction precipitating between 40 and 60% saturation was collected by centrifugation. It was then dissolved in a small volume of redistilled water and heated for 7 min in a water

bath kept at 58°. The precipitate formed was removed and the clear supernatant was brought to 60% saturation with respect to $(NH_4)_2SO_4$ The precipitate was collected by centrifugation and dissolved in 5 mM malonate buffer, pH 6 2, containing 10 mM 2-mercaptoethanol. Essentially the same procedure was employed for the purification of liver and muscle enzymes, except that the addition of $MgSO_4$ (10 mM) followed by acidification to pH 4 5 was employed in place of the protamine treatment The precipitate formed was removed and the supernatant was neutralized to pH 6.6 for further fractionation

*Assay methods*

At all stages of purification, fructose diphosphatase activity was determined at 22° by the spectrophotometric method of TAKETA AND POGELL[3] The standard assay mixture contained 50 mM Tris buffer, pH 7.4, 10 mM $MgSO_4$, 1 mM EDTA, 0 2 mM $NADP^+$, 5 $\mu$g each of phosphoglucose isomerase and Glc-6-*P* dehydrogenase and tissue extract or enzyme preparation in a final volume of 2 ml In order to obtain a maximum activity, assay mixture *minus* substrate was incubated for 5 (for extract) or 10 min (for purified enzyme) at 22° (ref 3). During this period, no reduction of $NADP^+$ was detected The reaction was then started by addition of Fru-1,6-$P_2$ (0.1 mM) and the reduction of $NADP^+$ was followed at 340 m$\mu$ in an Hitachi recording spectrophotometer. Except for tumor extracts which exhibited a lag period of almost 10 min in the onset of Fru-6-*P* formation (see RESULTS), readings began 1 min after the addition of the substrate and the enzyme activity was calculated from changes in extinction during the following 5 min. Especially in crude extracts, the rate of $NADP^+$ reduction increased slightly with time, presumably owing to the steady decrease of Fru-1,6-$P_2$ which is a specific inhibitor of fructose diphosphatase (see RESULTS) In crude extracts, the rate of reoxodation of NADPH was negligible In some instances, the reaction was terminated by trichloroacetic acid and the amount of inorganic phosphate released during the incubation was determined by the method of TAKAHASHI[6]. With purified tumor enzyme, essentially equal quantities of Fru-6-*P* and inorganic phosphate were formed from Fru-1,6-$P_2$ One unit of activity represents the formation of 1 $\mu$mole of Fru-6-*P* per min and specific activity is expressed as units per mg of protein.

In tumor extracts, the activity of 6-phosphogluconate dehydrogenase was much higher than that of fructose diphosphatase. For this reason, the fructose diphosphatase activity of tumor extracts was calculated on the basis of one-half of the NADPH equivalents formed.

*Other determinations*

ATP[7], ADP, AMP[8] and lactate[9] were determined as described in the references Protein content of enzyme preparations was estimated by the method of LOWRY *et al* [10]

*Chemicals and commercial enzymes*

ATP, ADP, AMP, Glc-6-*P* dehydrogenase and phosphoglucose isomerase were obtained from Boehringer Fru-1,6-$P_2$ was the product of Sigma (grade 98–100%) and its concentration was estimated by the method of BUCHER AND HOHORST[11].

RESULTS

*Fructose diphosphatase activity in extracts*

Fructose diphosphatase activity of Ehrlich ascites tumor extracts was studied under the assay conditions described by TAKETA AND POGELL[3] and using 0.1 mM Fru-1,6-$P_2$ as substrate. The time course of activity is recorded in Curve I of Fig 1*. There was a time lag of almost 10 min before the onset of Fru-6-*P* formation The lag was constantly observed with fresh extracts, but its duration varied considerably according to the treatment to which fresh extracts were subjected The lag period was

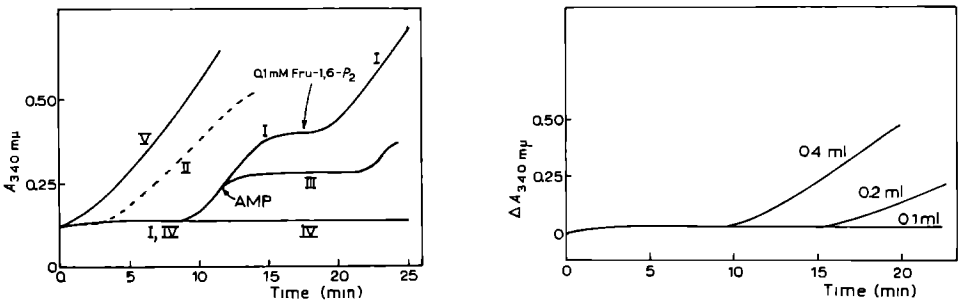



Fig 1 Spectrophotometric assay of fructose 1,6-diphosphatase activity in Ehrlich ascites tumor extract under the standard conditions described in text 0 4 ml of fresh extract was used At zero time, 0 1 mM Fru-1,6-$P_2$ was added and the reduction of $NADP^+$ was followed at 340 mμ. I, control, II, 5 mM ATP was added at zero time, III, 0 1 mM AMP was added at the arrow, IV, 0 1 mM iodoacetate was added prior to preincubation, and V, extract dialyzed against 1 mM EDTA (pH 7 0) overnight was used instead of fresh extract Before assay, $MgSO_4$ was added to the dialyzed extract to a final concentration of 10 mM and the precipitate formed was centrifuged off

Fig 2 Fructose 1,6-diphosphatase activity of Ehrlich ascites tumor extract at three different extract concentrations The assay conditions were the same as for the experiment depicted by Curve I of Fig 1, except that varying concentrations of extract were employed

reduced greatly by dialysis (Curve V) and prolonged by aging When 0 1 mM iodoacetate was included in the assay mixture, no Fru-6-*P* formation was noted during 25 min of incubation (Curve IV) This is not due to a direct inhibition of fructose diphosphatase by iodoacetate since iodoacetate did not suppress the enzyme activity in dialyzed extracts or purified preparation The duration of lag was also dependent upon the amount of extract employed. As shown in Fig 2, the use of a greater amount of extract resulted not only in a higher rate of Fru-6-*P* formation but also in a marked reduction in lag period. It is also to be noted that the addition of 5 mM ATP markedly reduced the lag period (Curve II of Fig 1) The addition of 0.1 mM AMP suppressed Fru-6-*P* formation (Curve III), as would be expected from the reported high sensitivity of fructose diphosphatase from various tissues to AMP[3,4,13–18] With liver or muscle extracts, Fru-6-*P* formation was evident after a lag of less than 1 min.

* Raising the concentration of Fru-1,6-$P_2$ to 1 mM reduced the rate of Fru-6-*P* formation by fructose diphosphatase considerably Under these conditions, however, the occurrence of another Fru-6-*P*-forming activity was noted This activity, appearing without lag, has been identified as being catalyzed by phosphofructokinase working in the reverse direction[1] UYEDA AND RACKER[12] also reported that phosphofructokinase was readily reversible when Fru-6-*P* was removed by coupling to phosphoglucose isomerase and Glc-6-*P* dehydrogenase.

TABLE I

FRUCTOSE DIPHOSPHATASE ACTIVITY OF MOUSE TISSUES

The activities were measured under the standard conditions described in the text and expressed as μmoles of substrate transformed/g fresh weight or ml packed tumor cells/h at 22°. The numbers of extract or pooled extract (muscle) analyzed are given in parentheses. For tumor and liver, the results are given as means ± S.E.M. For muscle, the results obtained are recorded.

| *Tissue* | *Fructose diphosphatase activity* |
|---|---|
| Ehrlich tumor (10) | 8.64 ± 0.96 |
| Liver (5) | 702 ± 36 |
| Leg muscle (2) | 6.12, 5.28 |

Even during the lag period, tumor extracts utilized Fru-1,6-$P_2$ for the formation of lactate (see Table III). In Curve I of Fig. 1, Fru-6-*P* formation ceased at 15–20 min of incubation. This is due to the exhaustion of substrate, since the addition of 0.1 mM Fru-1,6-$P_2$ immediately restored the Fru-6-*P* formation to the original rate. This portion of Curve I has been used for the calculation of fructose diphosphatase activity in Ehrlich ascites tumor cells. In Table I, these data are expressed on a basis of packed cell volume (ml) and compared with the data for liver and muscle extracts expressed on a wet weight basis. The tumor had higher fructose diphosphatase activity than skeletal muscle. When the enzyme activities of extracts were compared on mg protein basis, the tumor activity was about 2.5% of the liver activity.

*Purification*

For the tumor enzyme, representative data of the progress of purification are summarized in Table II. In this example, the degree of purification was almost 40-fold and the final product had a specific activity of 0.077 unit/mg protein. It should be noted that the lag was no longer apparent after the second step in the purification procedure. The liver enzyme was purified 26-fold to a specific activity of 3.5 units/mg protein. The muscle enzyme was purified 48-fold to a specific activity of 0.077 unit/mg protein. These purified preparations were free of phosphofructokinase activity, but the tumor and muscle enzymes were still contaminated with significant aldolase (EC 4.1.2.7) activity.

TABLE II

PURIFICATION OF FRUCTOSE DIPHOSPHATASE FROM EHRLICH ASCITES TUMOR CELLS

The activities were assayed under the standard conditions described in the text.

| *Step* | *Specific activity (units/mg)* | *Purification (×)* | *Recovery (%)* |
|---|---|---|---|
| 1 Extract | 0.002 | 1 | 100 |
| 2 Protamine supernatant | 0.004 | 2 | 80 |
| 3 $(NH_4)_2SO_4$, 40–60% | 0.014 | 7 | 70 |
| 4 Supernatant from heat treatment | 0.062 | 31 | 46 |
| 5 Second $(NH_4)_2SO_4$, 60% precipitate | 0.077 | 39 | 40 |

The purified tumor enzyme, in a solution containing 10 mM mercaptoethanol and 5 mM malonate, pH 6.2, retained its original activity after storage for 1 month at 2°.

*Effect of substrate concentration*

Fructose diphosphatase from various tissues is known to be inhibited by high concentrations of the substrate[3,4,13,14,16-19] The effects of Fru-1,6-$P_2$ concentration on the activities of purified fructose diphosphatases from different tissues of mouse are shown in Fig. 3 As in the case of rat liver enzyme[3], plots of activity against log[Fru-1,6-$P_2$] gave bell-shaped curves with all the enzymes tested and inhibition of the activities became evident with Fru-1,6-$P_2$ concentrations greater than 0 05 mM For the tumor enzyme, the inhibitions were 35 and 50% with 1 and 10 mM Fru-1,6-$P_2$, respectively. From the Lineweaver–Burk plots of the data obtained in the range of non-inhibitory substrate concentrations, the apparent $K_m$ for Fru-1,6-$P_2$ of the tumor enzyme was calculated to be 8.1 $10^{-6}$ M The corresponding values for the muscle and liver enzymes were 3 9 and 4 2 $10^{-6}$ M, respectively. Under comparable conditions, a slightly lower value of 2 7 $10^{-6}$ M has been obtained for the purified rat liver enzyme[3]

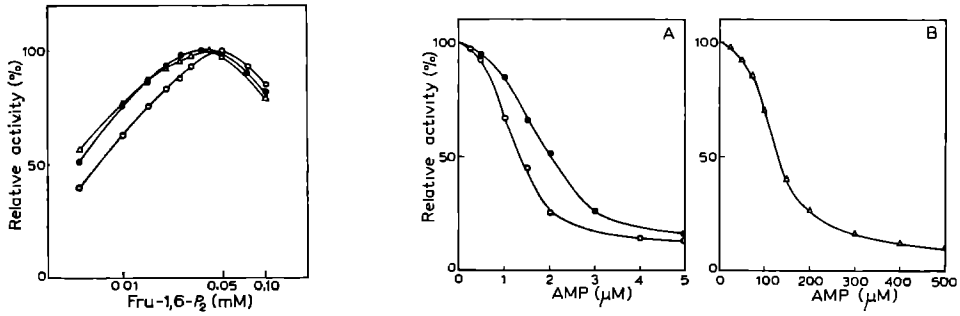
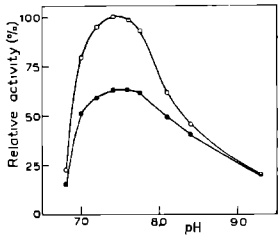



Fig 3. Effect of substrate concentration on fructose 1,6-diphosphatase activity The concentration is shown on the abscissa on a log scale. The activities of purified enzyme preparations from Ehrlich ascites tumor (○—○), mouse leg muscle (●—●) and mouse liver (△—△) were assayed under the standard conditions at Fru-1,6-$P_2$ concentrations shown.

Fig 4. Percentage inhibition of fructose 1,6-diphosphatase by varying concentrations of AMP. The activities of purified preparations were assayed under the standard conditions AMP was added just before the substrate A, Ehrlich ascites tumor (○—○) and mouse leg muscle (●—●). B, mouse liver (△—△)

*Inhibition by AMP*

Low concentrations of AMP inhibit the partially purified Ehrlich ascites tumor as well as muscle or liver fructose diphosphatase The inhibition was progressive with increasing concentrations of AMP and for all the preparations examined, plots of relative enzyme activity against AMP concentration gave sigmoid-shaped curves (Fig. 4) as in the case of the purified rat liver enzyme[3]. For the mouse liver enzyme, an inhibition of 50% requires an AMP concentration of 130 $\mu$M, a value comparable to that reported by TAKETA AND POGELL[3] for the purified rat liver enzyme For Ehrlich ascites tumor and mouse leg muscle enzymes, the values found are 1 3 and 2.1 $\mu$M, respectively Thus, the sensitivity of the tumor or muscle fructose diphosphatase to



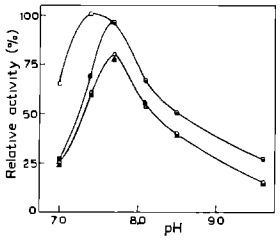




Fig 5 Effect of pH on the activity of purified fructose 1,6-diphosphatase from Ehrlich ascites tumor at 0 1 mM (○—○) and 1 mM (●—●) Fru-1,6-$P_2$ Buffers 50 mM triethanolamine, pH 6 6–8 4, 80 mM glycine, pH 9 3 Other assay conditions were as described in the text

Fig 6 Effects of EDTA and AMP on the activity of purified Ehrlich ascites tumor fructose 1,6-diphosphatase as a function of pH ○—○, control (with the buffers as described in Fig 5), ●—●, the addition of 1 (pH 7 0–7 7) or 2 $\mu$M (pH 8 1–9 3) AMP was followed by substrate, △—△, EDTA was omitted, and ▲—▲, EDTA was omitted and 1 (pH 7 0–7 7) or 2 $\mu$M (pH 8 1–9 3) AMP was added

AMP is 50–100 times that of the liver enzyme Such high sensitivities to AMP have also been found for fructose diphosphatase from cat[16] and frog muscle[14].

*Effect of pH*

At 0 1 mM Fru-1,6-$P_2$, the pH optimum for the purified tumor fructose diphosphatase was found to be 7.4 (Fig 5). Maximum inhibition by high substrate concentrations was also found at this pH At pH values greater than 9 0, there was no inhibition by 1 mM Fru-1,6-$P_2$ These properties are almost identical to the corresponding characteristics of the purified fructose diphosphatase from rat liver[3]

The omission of EDTA from the assay mixture resulted in changes in the pH dependence of Ehrlich ascites tumor fructose diphosphatase activity (Fig 6). The pH optimum was shifted to 7 7 and the activity at pH 7 4 was decreased to almost 60% of the maximum

As in the case of purified rat liver fructose diphosphatase[3], the extent of inhibition by AMP of the purified tumor enzyme decreased with increasing pH (Fig 6) At pH 7.7 and above, practically no inhibition could be detected by 1–2 $\mu$M AMP. In the absence of EDTA, 1–2 $\mu$M AMP caused no inhibition of enzyme activity between pH 7 0 and 9.4

*Effect of cations*

Fructose diphosphatase purified from Ehrlich ascites tumor requires $Mg^{2+}$ or

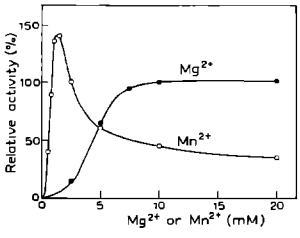



Fig 7 Effects of $Mg^{2+}$ or $Mn^{2+}$ concentration on the activity of purified Ehrlich ascites tumor fructose 1,6-diphosphatase The standard conditions were employed except that given concentrations of the divalent cations were present

$Mn^{2+}$ for activity. As shown in Fig. 7, maximum activity was attained at 1 5 mM $Mn^{2+}$ or 10 mM $Mg^{2+}$ Above 1 5 mM, increasing concentrations of $Mn^{2+}$ were progressively inhibitory to the enzyme activity.

*Lag period for fructose diphosphatase activity in tumor extracts*

When crude extracts from Ehrlich ascites tumor cells were examined for fructose diphosphatase activity, there was a time lag of almost 10 min in the onset of Fru-6-*P* formation (Curve I of Fig. 1). In these experiments, the zero-time assay mixture contained 20–40 $\mu$M AMP (derived from extracts), which was sufficient to suppress the fructose diphosphatase activity Dialyzed extracts (Curve V) or purified enzyme

TABLE III

CHANGES IN THE LEVELS OF ADENINE NUCLEOTIDES AND LACTATE DURING THE LAG PERIOD IN FRUCTOSE DIPHOSPHATASE ASSAY OF EHRLICH TUMOR EXTRACT

The fructose diphosphatase assay of tumor extract was conducted under the standard conditions in the absence and presence of iodoacetate For the time course of activity under these conditions, see Curves I and IV, Fig 1 At the times indicated, the reaction was stopped by the addition of perchloric acid and the amounts of adenine nucleotides and lactate were determined as described in the references (see text) The levels were expressed as m$\mu$moles/ml of the assay mixture

| *Iodoacetate (mM)* | 0 | 0 | 0 1 | 0 1 |
|---|---|---|---|---|
| *Duration of incubation (min)* | 0 | 10 | 0 | 15 |
| ATP | 17 | 83 | 16 | 9 |
| ADP | 59 | 37 | 48 | 44 |
| AMP | 46 | 5 | 49 | 78 |
| Lactate | 169 | 255 | 162 | 165 |

failed to exhibit such a time lag, but the latter was inhibited severely by extracts heated at 100° for 3 min. It is therefore apparent that during the lag period, fructose diphosphatase is inhibited by AMP contained in the extracts As shown in Table III, when iodoacetate was absent, there was a marked decline in the concentration of AMP throughout the lag period This decline in AMP appears to be responsible for the termination of lag, since, when iodoacetate was present, neither decline in AMP nor release of time lag could be observed (Table III and Curve IV of Fig 1). The ability of tumor extracts to remove AMP was also demonstrated by the fact that the inhibition of fructose diphosphatase by exogenous AMP was only transient (Curve III).

In a similar experiment, ATP added at zero time markedly reduced the lag period (Curve II) ATP appears to stimulate the removal of AMP through the mediation of adenylate kinase (EC 2 7 4.3) (see DISCUSSION).

## DISCUSSION

The present studies have demonstrated that the fructose 1,6-diphosphatase identified in extracts of mouse Ehrlich ascites tumor cells possesses similar properties to that found in rat liver[3] Like the rat liver enzyme, the tumor enzyme is inhibited by Fru-1,6-$P_2$ and AMP in a very specific manner One marked difference between the

two enzymes, however, concerns the sensitivity to AMP: The $K_i$* for AMP of the tumor enzyme is found to be 1 3 $\mu$M, a value much lower than the value of 100 $\mu$M reported by TAKETA AND POGELL[3] for the purified rat liver enzyme. The fructose diphosphatase from mouse liver studied in the present work also has a high $K_i$ (130 $\mu$M) comparable to that of rat liver enzyme. In contrast, the mouse skeletal muscle enzyme exhibited a $K_i$ value (2 1 $\mu$M) close to that of tumor enzyme. Such low $K_i$ values have also been obtained for the fructose diphosphatase from skeletal muscle of other species[14,16].

It appears that there are at least two different types of fructose 1,6-diphosphatase existing in mammalian tissues The enzymes from livers of rat and mouse and also the enzyme from bovine lactating mammary gland[18] are characterized by being comparatively insensitive to inhibition by AMP and may be designated as "Type L". Fructose diphosphatase purified from rat kidney exhibited a $K_i$ value of 180 $\mu$M and thus may also be a "Type L" enzyme (unpublished observation) The diphosphatases from skeletal muscles of various species can be included in the second group, "Type M", which is characterized by being extremely susceptible to inhibition by AMP. In this sense, the fructose diphosphatase from Ehrlich ascites tumor cells obviously is a "Type M" enzyme. At present, no information is available for possible molecular differences between the two types of enzyme.

Since the activities of "Type M" fructose diphosphatase are generally very low wherever found, those tissues which are characterized by the occurrence of the "Type L" enzyme, such as liver or mammary gland, may also contain the "Type M" enzyme. In this laboratory, fructose diphosphatase activity was determined in extracts of various strains of Yoshida ascites hepatoma[20]. Some of these strains were completely devoid of fructose diphosphatase activity, but other strains were found to contain only the "Type M" enzyme The "Type M" enzyme may be stable under the conditions of cancer progression as compared to the "Type L" enzyme Or the appearance of the "Type M" enzyme in certain liver tumors may be a consequence of carcinogenesis, under which liver-specific fructose diphosphatase is replaced gradually by unspecific "Type M" enzyme. This interpretation appears to be in line with the observation made by a number of workers that the carbohydrate metabolic features of rapidly growing hepatomas resemble those of the muscle[21–24]

As in the case of rat liver fructose diphosphatase[3], the degree of inhibition by AMP of the tumor enzyme is pH-dependent. At pH values greater than 7 7, there was no inhibition by 1–2 $\mu$M AMP (Fig 6) This behavior of the tumor enzyme may be explained if we assume the occurrence of two activities differing from each other in pH optimum, as has been noted for highly purified fructose diphosphatase from rabbit liver[25], *Candida utilis*[26] and *Polysphondylium pallidum*[27]. According to ROSEN *et al.*[26], the activity at neutral pH, observable only when EDTA is present, is highly sensitive to inhibition by AMP, whereas the activity at alkaline pH is rather insensitive. The addition of AMP or the omission of EDTA shifted the pH optimum of tumor fructose diphosphatase from 7 4 to 7 7 (Fig 6) Furthermore, no inhibition by AMP was observed in the absence of EDTA.

The physiological role of fructose diphosphatase in tumor tissues has not been elucidated In Ehrlich ascites tumor cells, the phosphofructokinase/fructose diphos-

* $K_i$ denotes concentration of inhibitor necessary for 50% inhibition

phatase activity ratio is almost 100 (ref. 28). We have previously reported that the oxidation of [6-$^{14}$C]glucose to $^{14}CO_2$ observed in Ehrlich ascites tumor cells in the presence of iodoacetate and methylene blue is due to the phosphogluconate oxidation of Glc-6-*P* resynthesized from pentose cycle-generated glyceraldehyde 3-phosphate[1] The oxidation thus requires the conversion of Fru-1,6-$P_2$ to Fru-6-*P* In the presence of iodoacetate and methylene blue, the cell levels of Fru-1,6-$P_2$, ADP and AMP are extremely high and those of Glc-6-*P* and ATP are markedly reduced. It was concluded that phosphofructokinase working in reverse rather than fructose diphosphatase is responsible for the conversion of Fru-1,6-$P_2$ to Fru-6-*P* (ref 1)

The occurrence of a lag period in the assay measurements of fructose diphosphatase activity in tumor extracts may explain why a previous study[2] failed to detect the enzyme activity in Ehrlich ascites tumor cells. The present studies revealed that during the lag period, the fructose diphosphatase in tumor extracts is inhibited by AMP. No such time lag could be observed for liver or muscle extracts. Although the mouse muscle fructose diphosphatase is also extremely sensitive to inhibition by AMP, muscle extracts contain AMP only in very small amounts. The AMP content of mouse liver extracts is as high as that of tumor extracts, but owing to the presence of a very active fructose diphosphatase, only very small amounts of liver extracts are required for assay. In addition, the liver enzyme is much less susceptible to inhibition by AMP than the tumor or muscle enzyme.

A considerable reduction in time lag was observed upon the addition of ATP (Fig 1). The addition of iodoacetate, on the other hand, not only inhibited lactate formation but also suppressed the removal of AMP (Table III) Rise in ATP, observed in the absence of iodoacetate, was also suppressed It appears that the removal of AMP depends upon the generation of ATP, which in turn depends upon glycolysis with Fru-1,6-$P_2$ as substrate. These results are consistent with adenylate kinase being responsible for the removal of AMP. Mediation of adenylate kinase was previously suggested by UNDERWOOD AND NEWSHOLME[4] for the ATP reversal of AMP inhibition observed with crude rat liver fructose diphosphatase preparation.

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