Purification and Properties of Galactokinase from Saccharomyces cerevisiae*

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Galactokinase (EC 2.7.1.6; ATP:o-galactose-1-phosphotransferase) was purified to homogeneity with a 50% yield from cells of Saccharomyces cerevisiae which were fully induced for the production of the galactose metabolizing enzymes. The purification was accomplished by: (a) ammonium sulfate fractionation, (b) streptomycin sulfate precipitation, (c) DEAE-cellulose chromatography, (d) hydroxylapatite chromatography, and finally (e) Bio-Gel A-0.5m gel filtration. The resulting preparation of galactokinase was judged to be at least 95% pure by the following criteria: (a) galactokinase-catalyzed phosphorylation of galactose was determined; the results showed the pH optimum of the reaction to be in the range of pH 8.0 to 9.0. The enzyme is highly specific for galactose since galactokinase did not appear to phosphorylate any of the other sugars tested at a rate greater than 0.5% of the rate of galactose phosphorylation. Amino acid analysis was performed on the enzyme preparation and the results were used to calculate the partial specific volume (b) of 0.736. The NH2-terminal sequence was determined for the first 3 residues. The molecular weight and subunit composition were determined by ultracentrifugation and polyacrylamide gel electrophoresis under dissociating and nondissociating conditions. The data obtained indicated that galactokinase is a monomeric protein of molecular weight 58,000.

Galactokinase (kinase) catalyzes the first reaction in the Leloir pathway which is used by most organisms including yeast to metabolize galactose (1, 2). The reactions in this pathway are:

\[
galactose + ATP \rightarrow \text{galactose-1-phosphate} + ADP
\]

Galactokinase has been purified from several sources, both prokaryotic and eukaryotic (3-6), and procedures giving a partial purification have been reported from yeast (7, 8). However, there is no report of a procedure yielding homogeneous enzyme from yeast, and the yeast enzyme has not been well characterized.

The galactose enzymes of yeast are of considerable interest, since their structural genes are linked, and are regulated by a complex control system involving both negative and positive elements (9-12). Such linkage of functionally related genes is rare in eukaryotes. In addition, it has been reported (13) that the galactose enzymes are complexed in vivo, yet there is no evidence for such a complex in cell extracts.

As the first step in a biochemical study of the regulation and interaction of the yeast galactose enzymes we have purified and characterized yeast galactokinase.

**EXPERIMENTAL PROCEDURES**

**Materials**—Saccharomyces cerevisiae strain X108D, a diploid resulting from the cross of the two haploids 108-3C (a, ga, ura1, trp1) and 478-5D (a, ga, his1) was kindly supplied by Dr. Bruce Adams, Department of Microbiology, University of Hawaii (12). The chemicals used were Bacto-tryptone (Difco Laboratories), yeast extract (BBL), triethanolamine (Eastman Organic), D-([14C]galactose (New England Nuclear; 0.05 mCi/0.17 mg), Omnipor (New England Nuclear), ammonium sulfate (Schwarz/Mann; enzyme grade), toluidine and glyceral (Mallinkrodt Chemical), DE81 anion exchange paper (Whatman), DEAE-cellulose (Whatman: DE52), hydroxylapatite (Clarkson Chemical Co.), Bio-Gel A-0.5m (Bio-Rad Laboratories), electrophoresis reagents (Bio-Rad Laboratories), mercuriethanesulfonic acid (Pierce Chemical; sequenase grade), and guanidine HCl (Schwarz/Mann; ultrapure), all other chemicals were purchased from Sigma Chemical Co.

**Growth of Cells**—Galactose-adapted cells of S. cerevisiae X108D were grown in a rich medium containing 2% Bacto-tryptone, 1% yeast extract, and 2% galactose at 30°C with moderate aeration. Cells were grown from an initial cell density of 107 cells/ml (A600nm = 1.2) to a final density before harvest of 109 cells/ml (A600nm = 12.0) in a 15-liter New Brunswick fermentor. Generation time under these growth conditions was approximately 9.5 h and the yield of cells was 10 g wet weight/liter.

**Assay**—Galactokinase was assayed by a modification of the procedure of Wilson and Hogness (4). The assay mixture contained 0.1 µmol of D-([14C]galactose (specific activity: 400 cpm/nmol), 0.16 µmol...
of ATP, 0.24 μmol of MgCl₂, 0.32 μmol of NaF, 0.1 μmol of dithioerythritol, and 10 μmol of triethanolamine/acetate, pH 8.0, in a total volume of 10 ml. The reaction was started by the addition of 0.01 to 0.10 enzyme units from a galactokinase solution obtained by dialyzing the sample to be assayed in a buffer containing 20 mM triethanolamine/acetate (pH 8.0), 1 mM EDTA, 1 mM dithioerythritol, and 0.1 mg/ml of bovine serum albumin. The assay mixture was incubated at 30° for 20 min and then 50 μl of the reaction mixture was spotted onto a strip (1.5 x 15 cm) of Whatman DE52 anion exchange paper 3 cm from the top of the strip. After drying at 110° the strip was eluted with H₂O by descending chromatography to separate unreacted galactose from the galactose 1-phosphate product until the water front had migrated 6 to 8 cm from the bottom of the spotting area. The strips were then dried at 110° and the original spotting area cut out and counted in 10 ml of toluene based scintillation fluid (4 g of Omniflor/liter of toluene). The amount of galactose 1-phosphate formed during the reaction was calculated from the specific activity of the [14C]galactose. One unit of enzyme activity is defined as the amount of enzyme that will catalyze the phosphorylation of 1.0 μmol of galactose/h under the conditions of the assay.

Protein determinations were made by a modification of the procedure of Lowry et al. (14) on samples precipitated with 5% trichloroacetic acid to avoid interference by dithioerythritol which was present in all buffers. Bovine serum albumin was used as the reference protein. All manipulations were performed at 0-4° unless otherwise indicated. All column materials were prepared according to manufacturer's instructions.

RESULTS

Purification Procedure for Galactokinase—A summary of the purification procedure data is presented in Table I.

Preparation of Crude Extract—Fifty grams of frozen or fresh cells (the initial specific activity of kinase in frozen cells was unchanged after 3 months of storage at -20°) were suspended in 100 ml of galactokinase buffer (20 mM triethanolamine/acetate (pH 8.0), 1 mM EDTA, 1 mM dithioerythritol). The suspension was passed through a French pressure cell (Amino Bowman) at 10,000 to 15,000 p.s.i. This extract was centrifuged at 20,000 × g for 15 min and the supernatant solution was decanted and saved. The pellet was resuspended in 100 ml of fresh galactokinase buffer and pressed and centrifuged as before. The supernatants from the two breakages were combined and diluted to 500 ml with galactokinase buffer (Fraction 1). This procedure resulted in 80 to 90% breakage of the yeast.

Ammonium Sulfate Fractionation—Ammonium sulfate, 133 g, was added to Fraction 1 over a period of 7 min while mixing thoroughly. After addition of the final aliquot of ammonium sulfate the mixture was stirred an additional 15 min, as before. The suspension was centrifuged as before and the supernatant was saved (Fraction 2). This fraction was 70 to 75% pure galactokinase as estimated from polyacrylamide gel electrophoresis and radioactivity measurements. However, at higher loading levels (75 pg/gel) two faint bands appeared which migrated slightly faster than galactokinase.

The buffer was changed once more and after 6 h the solution was dialyzed against 2.0 liters of 50 mM in ammonium sulfate by the addition of 0.90 ml of 1 M ammonium sulfate and then loaded onto the DEAE-cellulose column at a flow rate of 100 ml/h, collecting 15-ml fractions. The sample had entered the column when the A₂₈₀nm of the effluent was less than 0.04. Under these conditions galactokinase bound weakly to the column and was found in the wash through fractions, while 85% of the protein in the extract remained tightly bound to the column. Fractions of specific activity greater than 450 units/A₂₈₀nm were pooled and concentrated to 50 ml in an Amicon ultrafiltration apparatus containing a UM-10 membrane. The concentrated fractions were then dialyzed overnight against 1.0 liter of Buffer B (5 mM sodium phosphate (pH 6.8), 1 mM EDTA, 1 mM dithioerythritol, and 10% (v/v) glycerol). The dialysis buffer was then changed and dialysis continued an additional 6 h (Fraction 4).

Bio-Gel A-0.5m Gel Filtration—To remove the remaining impurities Fraction 5 was chromatographed on a Bio-Gel A-0.5m column equilibrated with Buffer A + 10 mM galactose (see Fig. 1 for details). The most efficient purification was obtained with samples smaller than 5 ml. The galactokinase eluted from the column as a sharp peak in the area of molecular weight 50,000 to 70,000. The fractions of highest specific activity were pooled and concentrated in the Amicon ultrafiltration apparatus to a final volume of 10 ml. The concentrated fractions were then centrifuged at 20,000 × g for 10 min and the supernatant was saved and frozen at -80° (Fraction 5). This fraction was 70 to 75% pure galactokinase as estimated from polyacrylamide gel electrophoresis and retained most of its activity after storage at -80° for over 1 year.

Bio-Gel A-0.5m Gel Filtration—To remove the remaining impurities Fraction 5 was chromatographed on a Bio-Gel A-0.5m column equilibrated with Buffer A + 10 mM galactose (see Fig. 1 for details). The most efficient purification was obtained with samples smaller than 5 ml. The galactokinase eluted from the column as a sharp peak in the area of molecular weight 50,000 to 70,000. The fractions of highest specific activity were pooled and stored at -80° (Fraction 6). Under these conditions of storage the purified enzyme lost less than 20% of its activity in 6 months of storage.

Analysis of Purity—The profile of galactokinase from the Bio-Gel A-0.5m column illustrated in Fig. 1 showed constant specific activity to within 5% for the pooled fractions. SDS-polyacrylamide gels (8%), run by the procedure of Weber and Osborn (15), showed a single band at a gel loading level of 25 μg/gel. However, at higher loading levels (75 μg/gel) two faint bands appeared which migrated slightly faster than galactoki-
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protein concentration and between different sets of gels. Addi-
banding pattern was not totally consistent. It varied with
decreased several bands appeared in the smeared area. The
half of the gel. As the amount of protein loaded on the gel was
rium.

At higher loading levels (40 pg/gel) only a large smear of
method of Davis (17) gave poor resolution and variable results.

Scan patterns of stained gels at 550 nm were similar to the
method of Crepeau et al. (19, 20) indicated a purity of at least

is the major protein present in fraction 6.

in several isozymic forms. These experiments are consistent
during electrophoresis or less likely yeast galactokinase exists
seems to indicate either an aggregation phenomena produced
alter this anomalous behavior.

Pairs of nondissociating gels were run together and one was
stained with Coomassie blue while the other was sliced into 2-
mM sections which were each crushed into 1 ml of kinase
buffer and let sit overnight and then the solutions were as-
sayed for kinase activity. Galactokinase activity always was
present in all areas that were stained with Coomassie blue.

Scan patterns of stained gels at 550 nm were similar to the
pattern of galactokinase activity in the parallel gels, however
the overall yield of kinase activity was only 5%. This behavior
seems to indicate either an aggregation phenomena produced
during electrophoresis or less likely yeast galactokinase exists
in several isozymic forms. These experiments are consistent
with all the other experiments which show that galactokinase
is the major protein present in fraction 6.

Analysis of fraction 6 by ultracentrifugation according to the
method of Crepeau et al. (19, 20) indicated a purity of at least
95% as estimated from the linear correlation coefficient of plots
of log $A_{280\text{nm}}$ versus radius$^2$ during sedimentation to equilib-
rrium.

Determination of Kinetic Constants—The $K_m$ values for the
two substrates of galactokinase, galactose and ATP, were
determined utilizing a modified assay procedure. To obtain the
$K_m$ for galactose, standard assay mixes were prepared with the
exception that the concentrations of ATP/Mg$^{2+}$ were raised to
5 times the $K_m$ level (as determined from preliminary experi-
ments). This ensures that the $K_m$ measurement for galactose
would be independent of the concentration of the other sub-
strate, ATP, since the ATP/Mg$^{2+}$ concentration was above
saturation during the reaction. Under these conditions the rate
of galactose phosphorylation was measured over a range of
different concentrations of galactose (0.5 to 8 mM). A con-
stant amount of purified galactokinase (0.045 unit, 19 ng,
Fraction 6) was added to each reaction mixture and the reac-
tions terminated before 10% of the galactose substrate had
been phosphorylated. This minimizes the effects of product
inhibition which was observed at higher levels of product. For
the determination of the $K_m$ for ATP the same basic procedure
was followed. Galactose was held constant at 5 times its $K_m$
level while the ATP concentration was varied during the rate
measurements (0.1 to 2.5 mM). The ATP/Mg$^{2+}$ ratio was always
held constant at 0.67 mol/mol. The data obtained were plotted
by the reciprocal method of Lineweaver-Burk (21). The data
plots are linear, indicating that under these conditions the
reaction follows standard Michaelis-Menten kinetics. The re-
ciprocal plots were analyzed using a linear regression, least
squares fitting program (22) on a Data General Nova Com-
puter. The computer analysis gave a $K_m$ value of 0.60 mM
for galactose and 0.15 mM for ATP. Standard deviation of the
values was less than 5% and gave a linear correlation coeffi-
cient of 0.97 (20,000 = $F$ ratio for slope). $V_{max}$ calculated from
the data was 57 pmol/h for 17 pg of galactokinase. This
corresponds to a turnover rate of the enzyme of 56 molecules of
galactose converted to galactose 1-phosphate/enzyme mole-
cule.

Determination of pH Optimum and Sugar Specificity—The
pH dependence of the galactokinase-catalyzed phosphoryla-
tion of galactose was investigated. The rate of phosphoryla-
tion of galactose by galactokinase (0.05 unit, 22 ng, Fraction 6) was
measured by the standard assay procedure utilizing different
buffer systems (100 mM) in the assay mix. 2-(N-morpho-
line)ethanesulfonic acid (Mesl/KOH was used for the pH
range 4.5 to 6.5, Tris/HCl for the range of 7.0 to 8.5, and
sodium carbonate/sodium bicarbonate for the pH 9.0 to 10.0 range.
The maximal rate of phosphorylation occurs in the pH range of
8.0 to 9.0. At pH values higher than 9.0 there is a very sharp
loss of activity while below pH 8.0 there is a more gradual
decline in the rate of phosphorylation down to pH 4.5. This is
consistent with the stability of the enzyme as measured by
dialysis since overnight dialysis of the enzyme at pH values
above 9.2 or below 5.5 results in loss of all enzymatic activity.

The specificity of the purified enzyme preparation for the
phosphorylation of galactose as compared to other structurally
similar sugars was investigated. Standard assays were set up
with the exception that [14C]galactose was reduced to a level of
half its $K_m$ value (0.30 mM). In addition various similar sugars
that were not labeled were added to the assay mixtures at final
concentrations of 21 mM. This results in a 70-fold excess of the
unlabeled sugar over galactose. Under these conditions it
would be expected that if the alternate sugar were phosphoryl-
ated to any reasonable extent, the apparent rate of galactose
phosphorylation would be markedly reduced. The sugars
tested were glucose, mannose, galactitol, arabinose, 2-deoxyga-
lactose, fucose, and lactose. None of these possible alternate

![Fig. 1. Gel filtration purification and analysis of fraction 5. Fraction 5 (3.5 ml) containing 21 mg of protein and 38,500 units was loaded onto a Bio-gel A-0.5 column (1.5 x 90 cm). The column was eluted with Buffer A = 10 mM galactose at a flow rate of 6 ml/h. Fractions of 2.5 ml were collected and assayed for galactokinase activity and protein concentration.](image-url)
substrates produced an inhibition of galactose phosphorylation greater than 5% under these conditions. This leads to the conclusion that these other sugars are not phosphorylated by galactokinase at a rate greater than 0.5% of the rate of galactose phosphorylation. Thus these data indicate that the yeast galactokinase is highly specific for galactose and in vivo galactose is probably the only substrate of the enzyme.  

**Amino Acid Analysis** Amino acid analysis was performed on Fraction 6 by the procedure of Spackman (23) utilizing a Beckman 120 C amino acid analyzer. The results of this analysis are shown in Table II, along with the amino acid composition of the Escherichia coli enzyme (26) for comparison. This amino acid composition was used to calculate the partial specific volume \( \theta \) (27). This value, which was used in the ultracentrifuge studies, was found to be 0.736.

**Molecular Weight Determination and Subunit Composition**—Molecular weight analysis and determination of the subunit composition of yeast galactokinase was performed by several techniques: SDS-polyacrylamide gel electrophoresis, equilibrium sedimentation ultracentrifugation, and sucrose density gradient centrifugation.

A molecular weight determination by polyacrylamide electrophoresis under dissociating conditions was performed on Fraction 6 using the procedure of Weber and Osborn (15) with 8% SDS polyacrylamide gels. In some determinations the samples were heated to 80° for 30 min and boiled 5 min in 1% SDS and 1% mercaptoethanol to assure complete dissociation. The sample contained 25 \( \mu \)g of galactokinase, 25 \( \mu \)g of bovine serum albumin \( (M_r = 68,000) \), 25 \( \mu \)g of yeast alcohol dehydrogenase \( (M_r = 37,000) \), and 25 \( \mu \)g of chymotrypsinogen \( (M_r = 27,000) \). The standards were run in parallel gels or in the same gel as the galactokinase with no difference in the results. Galactokinase migrated slightly ahead of the bovine serum albumin standard corresponding to a molecular weight of 60,000. Under these conditions galactokinase showed only one band. Sucrose density gradient centrifugation of crude extracts of yeast cells using the technique of Martin and Ames (28) indicated a molecular weight for galactokinase of approximately 55,000 in comparison with the standard proteins bovine catalase and yeast alcohol dehydrogenase (data not shown). The results of these two techniques indicate that the galactokinase molecule is a monomeric protein of molecular weight 57,000.

**Ultracentrifuge Analysis**—To confirm these results, purified galactokinase was analyzed by equilibrium sedimentation ultracentrifugation in a Beckman model E ultracentrifuge using the system and technique described by Crepeau et al. (19, 20). Runs at 4° and 20° at the concentrations of 0.5 mg/ml and 1.0 mg/ml were performed in 0.1 M sodium phosphate buffer, pH 7.0. All these conditions gave a molecular weight of 58,000 for galactokinase. The molecular weights observed were not affected by the addition of 10 mM galactose or 1 mM dithioerythritol. In all cases plots of \( 
\log A_{260} \) versus radius squared showed linearity indicating a one component system. Computer analysis of four determinations (6 cells/determination) gave a molecular weight of 58,000 for the yeast galactokinase.

Sedimentation equilibrium analysis of the enzyme in the presence of 6.0 M guanidine HCl gave variable results. The results were never consistent with dissociation of the enzyme into subunits and may have resulted from limited proteolytic degradation.

**Automated NH2-terminal Sequence Analysis**—Purified galactokinase (36 nmol) was sequenced on a Beckman 890 protein sequencer utilizing a double cleavage protein program (29). Identification of the phenylthiohydantoin amino acids was made by gas (30) and thin layer chromatography (31). The NH2-terminal tripeptide sequence was found to be (NH2-Thr-Lys-Ser-).

## DISCUSSION

A procedure for isolating galactokinase from *S. cerevisiae* in high yield (50%) to a purity greater than 95% has been developed. Approximately 50 mg of pure galactokinase can be obtained from 50 g wet weight of yeast cells. The enzyme in our hands is highly stable since we observe minimal loss of activity during most of the isolation procedure. Protease activity was not a major problem during the isolation of this yeast enzyme as it has been with some other yeast enzymes. The ease of obtaining large amounts of yeast galactokinase by this procedure makes the enzyme suitable for studies requiring large amounts of purified enzyme.

From the final specific activity of the purified galactokinase (2300 units/mg) and the initial specific activity in crude extracts (35 units/mg) one can estimate that this protein accounts for approximately 1.5% of the soluble protein in yeast cells growing on galactose. If the other galactose metabolizing enzymes in the cell (transferase and epimerase) are made in equal amounts as suggested by genetic experiments (9-11) one can estimate that the enzymes of the galactose operon in yeast constitute about 5% of the total soluble protein. This would make the transcription and translation of the galactose operon structural genes a relatively active process during growth on galactose. This high activity of the galactose operon makes the system amenable to further studies concerning the nature of eukaryotic gene expression and its regulation.

Ultracentrifugation experiments on the purified enzyme, sucrose gradient centrifugation, and elution position during gel filtration under conditions where the enzyme is fully active

### Table II

| Amino acid | Residues/molecule (Saccharomyces cerevisiae) | Mole % |
|------------|---------------------------------------------|-------|
| Lysoleic acid | 45.1 | 8.7 | 4.3 |
| Histidine | 11.6 | 2.2 | 1.8 |
| Arginine | 10.7 | 2.1 | 3.5 |
| Tryptophan | 5.2 | 1.0 | 0.5 |
| Aspartic acid | 50.9 | 9.5 | 10.0 |
| Threonine | 26.2 | 5.0 | 3.3 |
| Serine | 42.7 | 8.2 | 3.7 |
| Glutamic acid | 58.2 | 11.2 | 13.9 |
| Proline | 30.3 | 5.8 | 3.9 |
| Glycine | 25.6 | 4.9 | 9.0 |
| Alanine | 45.3 | 8.7 | 10.7 |
| Cysteine | 10.9 | 2.1 | 2.8 |
| Valine | 39.4 | 7.6 | 9.8 |
| Methionine | 8.7 | 1.7 | 2.7 |
| Isoleucine | 20.0 | 3.8 | 6.0 |
| Leucine | 45.8 | 8.8 | 8.3 |
| Tyrosine | 19.2 | 3.7 | 2.5 |
| Phenylalanine | 24.9 | 4.6 | 3.5 |

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Ultracentrifugation experiments on the purified enzyme, sucrose gradient centrifugation, and elution position during gel filtration under conditions where the enzyme is fully active
indicate a molecular weight for the intact native enzyme of 58,000. Treatment of this enzyme under conditions which dissociate most proteins (i.e., heating in the presence of 1% SDS and 1% β-mercaptoethanol) and electrophoresis on SDS-polyacrylamide gels gave the same molecular weight value. Although guanidine HCl treatment in the presence of heat altered the molecular weight of the enzyme as judged by sedimentation equilibrium ultracentrifugation, the data could not be fitted by a model involving dissociation into subunits. NH₂-terminal sequence analysis of the purified enzyme showed only a single sequence and the yield of less than 1 residue/molecule for each cycle of the degradation is best fitted by the existence of a single polypeptide chain. The bulk of the experimental data seems to support the conclusion that the yeast galactokinase is a monomeric protein of molecular weight 58,000 molecular weight. This is in contrast to the report of Rustum and Bernard (8) who reported the S. cerevisiae galactokinase to be a protein of molecular weight 100,000 composed of four subunits of molecular weight 23,000. We did not obtain any data to support this conclusion.

Comparison of the molecular and catalytic properties determined for the yeast enzyme with those of galactokinases isolated from other sources both prokaryotic and eukaryotic does indicate similarities between the different enzymes. A summary of this data is shown in Table III. It is evident that galactokinase from most sources is a monomeric protein (e.g., E. coli, pig liver). The human red blood cell enzyme was reported to partially dissociate only after “aging.” The 60,000 molecular weight of the “native” enzyme seems to be characteristic of the eukaryotic form since it was observed in all eukaryotic sources.

In catalytic properties the yeast enzyme seems to resemble very closely the prokaryotic E. coli enzyme. The \( K_m \) values for the two substrates, galactose and ATP, are very similar for the yeast and E. coli enzymes, and the catalytic rate of the yeast enzyme is about 80% of the catalytic turnover rate of the E. coli enzyme. The \( K_m \) values for ATP of galactokinase from all sources are quite similar (i.e. in the 0.1 to 0.3 mM range). However the \( K_m \) of the yeast enzyme for galactose is about 3 to 5 times higher than the pig liver and human red blood cell enzymes. The catalytic rates of galactose phosphorylation in these cases however are similar. All galactokinases seem to have a pH optimum in the range of pH 6.0.

The yeast enzyme is very similar to the E. coli in catalytic properties, but has a molecular weight 1.5 times higher. We found no cross-reaction between purified yeast galactokinase and antisera against the purified E. coli enzyme; nor did we find any cross-reaction between the purified E. coli enzyme and antisera against purified yeast galactokinase (using immunodiffusion; data not shown). It remains to be determined as to the extent of similarity as measured by homology of sequences of the yeast and E. coli enzymes. However the similarity between the prokaryotic and simple eukaryotic enzymes may be significant.

**Acknowledgments**—We are indebted to Dr. Stuart Edelstein and Dr. Richard Crepeau for assistance in performing the ultracentrifuge studies and to Dr. David Schlesinger for performing the sequencing of the NH₂-terminal residues of galactokinase. We would also like to acknowledge Dr. Gerald Fink for his advice and comments.

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**Table III**

Properties of purified galactokinases

| Source                  | Galactone | ATP     | Subunit size | Molecular weight | pH optima |
|-------------------------|-----------|---------|--------------|------------------|-----------|
| Saccharomyces cerevisiae (this study) | 0.60      | 0.15    | No subunits  | 58,000           | 8.3       |
| E. coli (3, 4)          | 0.70      | 0.10    | No subunits  | 40,000           | 8.0       |
| Pig liver (5)           | 0.20      | 0.20    | No subunits  | 60,000           | 7.8       |
| Human red blood cell (6) | 0.12      | 0.35    | 26,000       | 55,000           | 7.9       |
| S. cerevisiae (8)       | 23,000    | 100,000 |              |                  |           |
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J. Biol. Chem. 1977, 252:1162-1166.

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