Nucleoside Triphosphate–Nucleoside Diphosphate Transphosphorylase (Nucleoside Diphosphokinase). II. Physical Properties of the Crystalline Enzyme from Brewers' Yeast*

Robert H. Yue, Robert L. Ratliff,† and Stephen A. Kuby

ABSTRACT: Several physical properties of the crystalline nucleoside diphosphokinase from yeast have been explored over a wide range of conditions: these include its sedimentation velocity, sedimentation equilibrium, translational diffusion, and electrophoretic behavior. No evidence for any gross heterogeneity in the protein preparation was found by these techniques. The observed sedimentation coefficients (at pH values from 5.6 to 7.9 at 3°) are a function of the protein concentration, whereas the diffusion coefficients show only a slight dependency. The measured weight-average molecular weights by "approach to equilibrium" and by true sedimentation equilibrium techniques are also concentration dependent. By suitable extrapolations to infinite dilution, satisfactory agreement in molecular weights (*i.e.*, $M_{s,D}$ and M_{equil}) are obtained and a value of 102,000 ± 2000 g/mole (at zero protein concentration) is taken as the best value for its kinetic unit. A positive second virial coefficient could be estimated from the limiting slopes of $1/M_{w\,app}$ *vs.* C_m, *viz.*, 5×10^{-5} mole ml/g² at pH 5.9, 0.16 (Γ/2), 3°. Electrophoretic mobilities as a function of pH at several fixed ionic strengths show a significant influence of ionic strength on the apparent isoelectric state. The hypothetical isoelectric point at zero ionic strength is estimated to be *ca.* pH 8.0. A plausible model for this protein is suggested and a number of physical properties are summarized, *e.g.*, values of $s^0_{20,w}$ and $D^0_{20,w}$ for pH 5.6 (the apparent isoelectric point at (Γ/2) = 0.15) are 5.67×10^{-13} sec and 5.63×10^{-7} cm²/sec, respectively.

The isolation of the crystalline enzyme, nucleoside triphosphate–nucleoside diphosphate transphosphorylase (nucleoside diphosphokinase),[1] from brewers' yeast was reported in the first paper (Ratliff *et al.*, 1964) of this series. Since that time there appear to have been additional efforts to purify this enzyme from other sources including calf thymus (Nakamura and Sugino, 1966) and human erythrocytes (Mourad and Parks, 1966). However, although some kinetic studies (Mourad and Parks, 1966; Nakamura and Sugino, 1966) and substrate specificity studies on several preparations from different sources have been reported (Chiga and Plaut, 1962; Chiga *et al.*, 1963; Ratliff *et al.*, 1964; Nakamura and Sugino, 1966; Mourad and Parks, 1966; Goffeau *et al.*, 1967) and although a phosphorylated protein has been implicated as an catalytically active intermediate in partially purified preparations (Mourad and Parks, 1965; Norman *et al.*, 1965), there are apparently no reported systematic studies on the physicochemical properties nor on the homogeneity of these several preparations (for a review, consult also Weaver, 1962).

This report will deal in particular with an investigation of some physical properties of the isolated crystalline enzyme protein from yeast; a preliminary report of this work has been given elsewhere (Yue *et al.*, 1965). Later studies will be concerned with an elucidation of its subunit structure and amino acid composition; and finally it is hoped to provide a comparison of its physical and chemical properties and of its catalyzed reaction with two other types of ATP transphosphorylases[2] (ATP–AMP transphosphorylase (Noda and Kuby, 1957) and ATP–creatine transphosphorylase (Kuby *et al.*, 1954)), which are currently under study in this laboratory, in order to shed some light on the basic problem of ATP transphosphorylation.

Materials and Methods

Materials. Crystalline nucleoside triphosphate–nucleoside diphosphate transphosphorylase was isolated from brewers' yeast by the procedure described by Ratliff *et al.* (1964). Reagent grade Tris was of Sigma origin. All other reagents were the best available analytical grade commercial products. Twice-distilled deionized water was used in the preparation of all solutions for the physical measurements (when necessary, any dissolved traces of CO_2 in the water were removed by boiling).

* From the Laboratory for Study of Hereditary and Metabolic Disorders and the Departments of Biological Chemistry and Medicine, University of Utah, Salt Lake City, Utah. *Received June 2, 1967.* This work was supported in part by grants from the National Institutes of Health and the National Science Foundation.

† Permanent Address: Los Alamos Scientific Laboratories, University of California, Los Alamos, N. M.

[1] IUB systematic name: ATP:nucleosidediphosphate phosphotransferase.

[2] Abbreviations used: AMP and ATP, adenosine mono- and triphosphates.

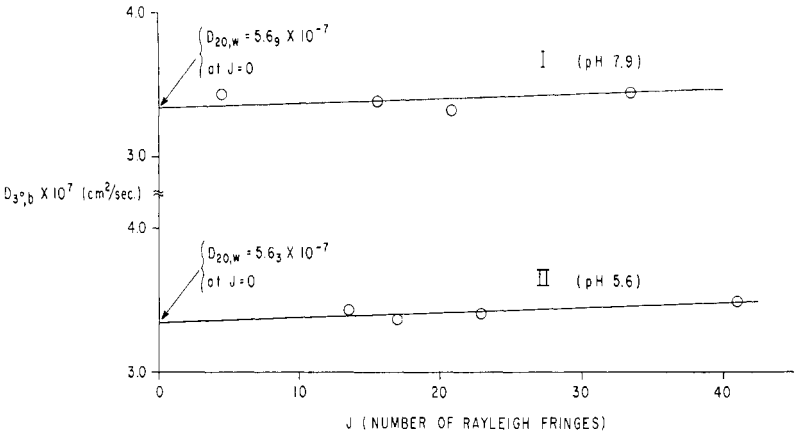

FIGURE 1: Diffusion coefficients of nucleoside triphosphate–nucleoside diphosphate transphosphorylase as a function of protein concentration at 3.00°. Data were obtained in both the 11-ml and 2-ml modified Tiselius cells. The concentrations have been expressed in terms of Rayleigh fringes (J) across the protein boundary, corrected for differences in optical paths of the two cells and referred to that of the 2-ml cell. Curve I: in 0.15 M KCl–0.01 M Tris (pH 7.9); $D_{3°,b} = (3.34 + 0.0029J) \times 10^{-7}$ cm²/sec. Curve II: in 0.141 M KCl–0.009 M acetate, ($\Gamma/2$) = 0.15, pH 5.6; $D'_{3°,b} = (3.35 + 0.0033J) \times 10^{-7}$ cm²/sec.

Diffusion. Free diffusion coefficients (D) were determined with the use of a Spinco Model H electrophoresis diffusion instrument at 3.00°, with the light source filtered to yield 546 mμ. Both the modified 11-ml standard electrophoresis cell and the modified 2-ml microelectrophoresis cell were employed. The two extension tubes were connected by side arms (Schachman, 1957) and the experimental procedure and method of boundary sharpening followed that described by Schachman (1957). Diffusion coefficients were calculated from photographs (on metallographic plates) of the Rayleigh interferograms. Computations were made by the method of Longsworth (1952).[3] The measured values of diffusion coefficient so obtained (D'_{meas}) were then corrected for the uncertainty in the "zero time" by means of the expression $D'_{meas} = D[1 + (\Delta t/t'_{meas})]$, where D is then $D_{3°,b}$, for 3° and buffer (Longsworth, 1947).

To convert $D_{3°,b}$ to "standard conditions," the Stokes–Einstein relation was employed together with the use of measured values of viscosity to yield $D_{20,w}$. Apparent weight-average molecular weights, from diffusion, sedimentation velocity (see below), and $\bar{V}_{app}$ data, were then obtained from the Svedberg relation, $M_{s,D} = RTS/D(1 - \bar{V}\rho)$. Since Gosting and Morris (1949) have found very slight inherent errors in the Stokes–Einstein relation, calculations of $M_{s,D}$ were made from the $s_{3°,b}$, $D_{3°,b}$, and $\bar{V}_{app\ 3°}$ sets of data, extrapolated to zero protein concentration (*i.e.*, $s^0_{3°,b}$ and $D^0_{3°,b}$).

To extrapolate diffusion coefficients to zero concentration (D^0), theoretically, the appropriate value of the concentration should be the site in the boundary from which the actual measurements are made (Gosting, 1956), *e.g.*, $C_0/2$. However, and as will be shown, the concentration dependency of $D_{3°,b}$ values proved to be so slight (*e.g.*, see below, Figure 1) that only insignificant errors are introduced in the evaluation of $D^0_{3°,b}$ at zero protein concentration by the use of plots of $D_{3°,b}$ *vs.* the measured Rayleigh fringes (J) across the protein boundary. To evaluate nonideality factors, however, the slopes of such plots may incorporate slight errors.

Sedimentation Velocity. Sedimentation velocities were measured at 3° in a Spinco Model E analytical ultracentrifuge, equipped with a RTIC temperature-control system and a phase plate as a schlieren diaphragm (the schlieren optical system was aligned according to Gropper, 1964). Kodak metallographic plates were used to photograph the schlieren patterns with a Kodak 77A filter over the light source. Sedimentation coefficients at 3° and buffer ($s_{3°,b}$) were calculated in the usual way from log x *vs.* t plots (Schachman, 1957) and converted to $s_{20,w}$ values with the use of measured values for the densities and viscosities of buffers employed and values for the partial specific volume of the protein (Svedberg and Pedersen, 1940). The sedimentation coefficient at infinite dilution was obtained by extrapolation from a plot of the apparent sedimentation coefficients and the average boundary concentration (*e.g.*, *cf.* Schachman, 1959; Jullander, 1945; Kegeles and Gutter, 1951).

From the treatment of Goldberg (1953) it is known that it is the second moment of the gradient curve which provides a measure of the sedimentation velocity of individual molecules, but for symmetrical boundaries without too large a degree of boundary spreading by diffusion (*e.g.*, see Figure 2, below) use of the maximum ordinate can be justified and taken as accurate indication of individual particle movement in the plateau region (*e.g.*, Schachman, 1959). In plots of s *vs.* c to evaluate s^0 (at $c \rightarrow 0$) according to the Goldberg theory, the

[3] The Tables of Probability Functions employed in the calculation were originally obtained from the Federal Works Agency, Superintendent of Documents, Washington, D. C. (1941).

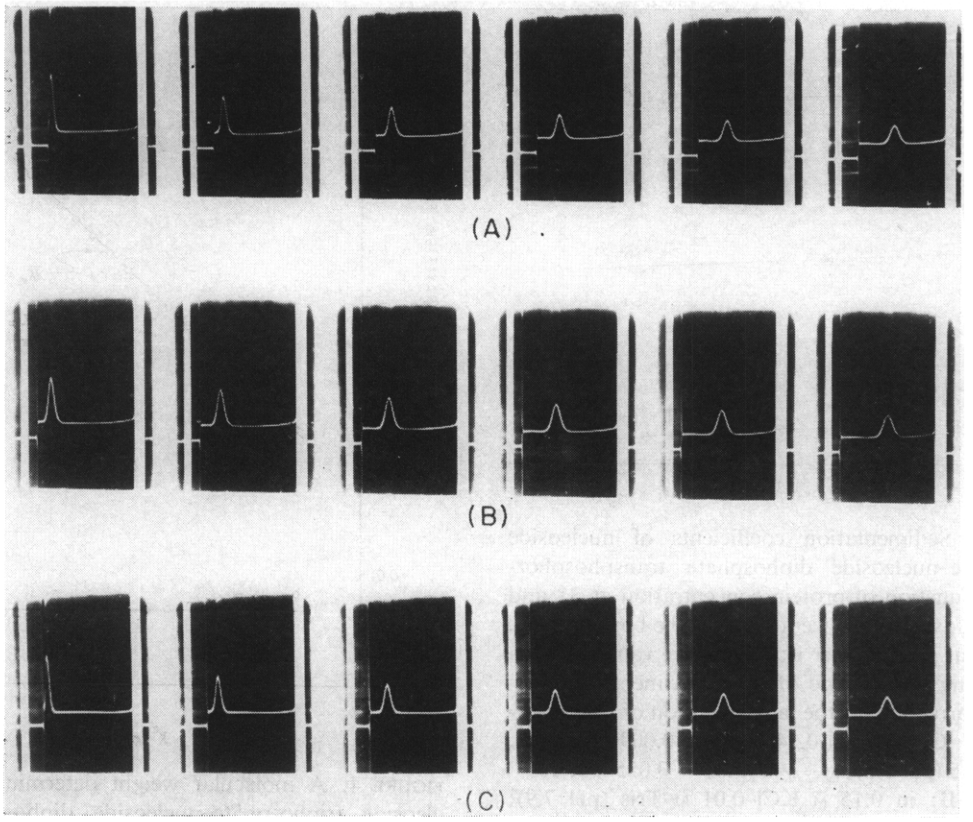

FIGURE 2: Sedimentation velocity patterns of nucleoside triphosphate–nucleoside diphosphate transphosphorylase sedimentation proceeds to the right at 16-min intervals; 59,780 rpm at 3.0–3.5°. The schlieren diaphragm (phase plate) angle was set at 75°. (A) In 0.141 M KCl–0.009 M acetate, ($\Gamma/2$) = 0.15 (pH 5.6) with an initial protein concentration of 5.70 mg/ml. (B) In 0.15 M KCl–0.01 M phosphate (K^+) (pH 6.8) with an initial protein concentration of 6.92 mg/ml. (C) In 0.15 M KCl–0.01 M Tris (pH 7.9) with an initial protein concentration of 5.99 mg/ml.

appropriate value of c should therefore correspond to that in the plateau region; otherwise there are several refined methods available which permit an accurate extrapolation of s, either at zero time or for its evaluation during the run (*e.g.*, Trautman *et al.*, 1954; Alberty, 1954; Baldwin, 1957). One simple and practicable method which permits an accurate extrapolation for those cases where the concentration dependency is not unusual (provided the temperature is controlled, see below) makes use of the average concentration between first and last exposures during the run (*e.g.*, Jullander, 1945; Kegeles and Gutter, 1951; Schachman, 1959). The usefulness and precision of this essentially empirical method is to be justified in the final analyses of s^0 (*e.g.*, see below Figure 3) and $M^0_{s,D}$. However it should be realized that this type of plot may impart small errors in the estimation of nonideality coefficients if evaluated from the slopes of sedimentation velocity and diffusion data, but these coefficients are more correctly evaluated from thermodynamic means (Schachman, 1959), *e.g.*, by sedimentation equilibrium methods (see below).

Ehrenberg's (1957) Adaptation of the Archibald Approach to Sedimentation Equilibrium Procedure. All experiments were performed at 3.0° in a Spinco Model E analytical ultracentrifuge using schlieren optics. A single-sector synthetic boundary cell of the valve type and 12-mm optical path was employed. The experimental procedure followed that described by Ehrenberg (1957), with slight modifications. The cell was filled with 400 μl of protein solution and the approach to sedimentation equilibrium was performed. Photographs were taken at 8-min intervals. The solution in the cell was then carefully mixed and 250 μl of buffer was added to the cup. The rotor was then accelerated to the equilibrium speed to give C_0. The cell was then emptied of its contents and carefully cleaned, without dismantling, and it was filled with 400 μl of buffer to provide the appropriate base line for subsequent calculations. Apparent weight-average molecular weights were determined by the Archibald (1947) equation for the meniscus, $M_{w,m} = [RT(dc/dx)_m]/[(1 - \bar{V}\rho)\omega^2 x_m C_m)]$, in which $M_{w,m}$, R, T, ρ, ω, and $\bar{V}$ have their usual meaning and C_m and $(dc/dx)_m$ are the concentration and the concentration gradient at the meniscus position (X_m), respectively. The schlieren patterns were enlarged nine times and were traced on a graph paper, and the base line taken at the appropriate time was superimposed on it. The areas were then measured with a planimeter. The exact ordinate position of the meniscus was ascertained

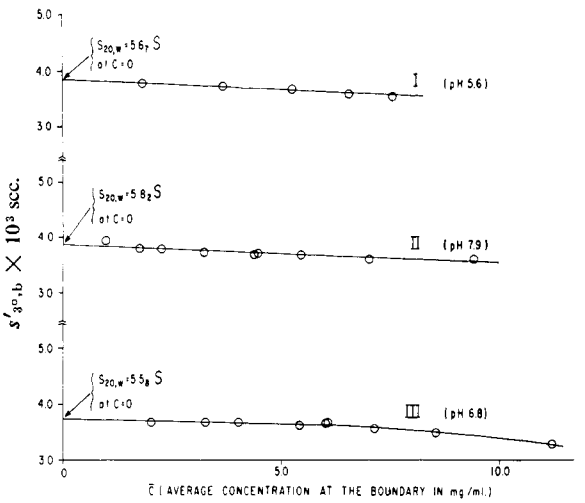

FIGURE 3: Sedimentation coefficients of nucleoside triphosphate–nucleoside diphosphate transphosphorylase as a function of protein concentration at 3° and pH 5.6, 7.9, and 6.8. Concentration at the boundary (C) in milligrams per milliliter is the average value between the beginning and the end of the experiment, as determined by correction for the radial dilution of the sector-shaped cell. Curve I: in 0.141 M KCl–0.009 M acetate, ($\Gamma/2$) = 0.15 (pH 1.6); $s'_{3°,b} = (3.85 - 0.035c) \times 10^{-13}$ sec. Curve II: in 0.15 M KCl–0.01 M Tris (pH 7.9); $s'_{3°,b} = (3.87 - 0.033\bar{c}) \times 10^{-13}$ sec. Curve III: in 0.15 M KCl–0.01 M phosphate (K^+) (pH 6.8); $s'_{3°,b} = (3.76 - 0.0028\bar{c} - 0.0037\bar{c}^2) \times 10^{-13}$ sec.

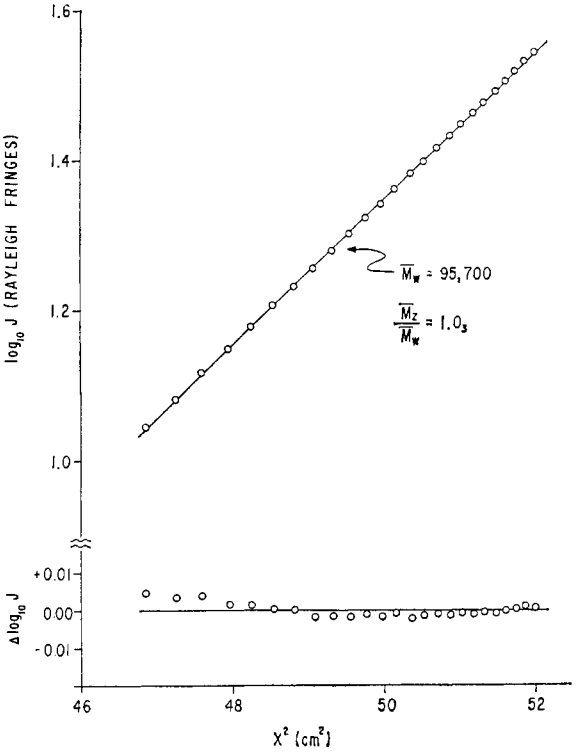

FIGURE 4: A molecular weight determination of nucleoside triphosphate–nucleoside diphosphate transphosphorylase from a sedimentation equilibrium experiment, expressed in terms of a plot of log J (log of the protein concentration in Rayleigh fringes) *vs.* x^2 (square of the distance, in centimeters squared, from the axis of rotation). The initial concentration of protein was 5.0 mg/ml in 0.0884 M KCl–0.0092 M KH_2PO_4–0.0008 M K_2HPO_4 (pH 5.9), ($\Gamma/2$) = 0.100 at 3.0°. The protein was sedimented initially at 11,250 rpm for 3.5 hr and then the speed was reduced to 6166 rpm. The time for the data given here was 39 hr after the equilibrium speed (6166 rpm) was reached at 3.0°, and no significant differences were noticed between 16.5 and 39 hr. A calculated value of M_{equil} (or $\bar{M}_w$) = 95,700 is obtained from these data and $\bar{M}_z/M_w$ = 1.03. For the lower "deviation" plot $\Delta(\log J)$ is the difference, at a given value of x^2, between the least-square straight line and the experimental points.

experimentally from experiments with ribonuclease whose weight-average molecular weight was found to be 13,800 by true sedimentation equilibrium (Yue *et al.*, 1967).

For experiments at various concentrations of protein, the values of $M_{w,m}$ were evaluated at the mensicus and correlated with their respective meniscus concentrations (see below). In the Ehrenberg (1957) approach extrapolations to the meniscus are of sufficient accuracy, provided inherent optical errors are recognized (LaBar, 1966). A plot of $1/M_{w,m\ app}$ *vs.* c_m provides a suitable extrapolation to $c \rightarrow 0$ (Kegeles *et al.*, 1957; Mueller, 1964), in which region the activity coefficient term disappears.

Sedimentation equilibrium experiments were performed also at 3° with the use of a rotatable light source and Rayleigh interferometric optics (Richards and Schachman, 1959); the optics were aligned also according to Gropper (1964). A slow stream of air passed over the outside of the lower collimating lens to eliminate moisture condensation during periods of centrifugation (Yphantis, 1964). The experimental procedure followed that described (Spinco Manual, 1964) with slight modifications (Yue *et al.*, 1967). To reduce the time required to reach sedimentation equilibrium, the rotor was overspeeded for a calculated period of time followed by reduction in speed to the desired equilibrium speed (Hexner *et al.*, 1961), which was selected to provide a three- to fourfold ratio in (C_b/C_m), concentration at the cell bottom to that at the meniscus (Svedberg and Pedersen, 1940). The legends to the figures give the pertinent information.

The apparent weight-average molecular weights at various positions in the cell at equilibrium, were calculated from ln J_x *vs.* x^2 plots, according to $M_{wx} = \{[2RT]/[(1 - \bar{V}\rho)\omega^2]\}\{d(\ln c_x)/d(x^2)\}$ (where c_x was expressed in J_x fringes, and the other symbols bear their usual meaning); and the mean z-average molecular weight for the entire cell contents was calculated from the limiting slopes of the ln J_x *vs.* x^2 plots at the cell bottom and meniscus and with the use of the equation

$\bar{M}_z = (M_{wx_b}C_b - M_{wx_m}C_m)/(C_b - C_m)$ (Svedberg and Pedersen, 1940).

To explore the effects of the protein concentration on the molecular weight estimated by true sedimentation equilibrium runs, measurements were made over the comparatively narrow range permitted by this interferometric technique (*ca.* 1–5 mg/ml of initial protein concentrations, *i.e.*, C_0). For the most part, plots of $\ln J_x$ *vs.* x^2 proved to be essentially linear (*e.g.*, see below Figure 4, and where $\bar{M}_w = \bar{M}_z$) and which appeared to justify the use of calculated values of the weight-average molecular weights for the entire cell contents (*i.e.*, $\bar{M}_w$) in the evaluation of $\bar{M}_w^0$ (*i.e.*, at $c \rightarrow 0$), since the trend was small in the dilute protein concentration range. These data will be summarized later in a plot designed to correlate them with those values of $M_{w,m}$ obtained from Ehrenberg runs. A suitable extrapolation to $c \rightarrow 0$ could be obtained when $1/\bar{M}_w$ was plotted *vs.* C_0, and a good fit of these data could be made to all of the $1/\bar{M}_{w,m}$ data plotted *vs.* C_m obtained from the Ehrenberg runs. Another and theoretically sound approach is that of Van Holde and Baldwin (1958) for a two-component nonideal system, where the appropriate concentration to use corresponding to the weight-average molecular weight is that of $(c_b + c_m)/2$. However, its evaluation from the present data would appear to have the same relative degree of accuracy as that of a comparison of $\bar{M}_w$ with $\bar{M}_z$, and the most consistent and accurate approach in present case appeared to be the use of $1/\bar{M}_w$ with C_0 (see below), where the limiting slope is small.

Refractive Index Increments. Coincident with these studies, the Model H instrument was employed as Rayleigh interferometer to measure the refractive index increment of the protein at 546 mμ. The optical path of the cell was determined with a sucrose solution, with the use of the refractive index increments estimated from the data of Gosting and Morris (1949) [*i.e.*, $(\Delta n/\Delta c)_{3°} = 1.4653 \times 10^{-3}$], and the following equation $l = J\lambda/[c(\Delta n/\Delta c)]$ (where l is the optical path in centimeters; λ, the wavelength in centimeters; c, the concentration in g/100 ml; J, the differential number of Rayleigh fringes; and $(\Delta n/\Delta c)$, the refractive index increment of the solution in $(g/100\ ml)^{-1}$.

Electrophoresis. Electrophoretic (moving boundary) measurements were conducted in the Spinco Model H instrument at 1.0° with the 2-ml micro-Tiselius cell and schlieren optics. Solutions of protein were exhaustively equilibrated by dialysis 24–36 hr against buffers at various pH values and ionic strengths (see legend to Figure 5). Conductivity measurements at 0.0° were made on the equilibrated buffer, with the use of a Radiometer conductivity meter type DCM 2d and a refrigerated bath (Precision Scientific Instruments, Temptrol bath). The calculated electrophoretic mobilities are consequently those for 0° (Tiselius, 1937). Migration of the boundaries was measured from the center line of the boundary with a microcomparator (Gaertner) and calculation of mobilities was made in the conventional manner (Abramson *et al.*, 1942; Longsworth, 1942) on the descending boundary. To minimize deviation from ideality and convective disturbances in the cell, relative dilute protein concentrations (which range 2–5 mg/ml) and comparatively low electric field strengths (which were never higher than about 4 v/cm) were employed for 0.15 ionic strength buffers (and the 2-ml cell). "False boundaries" were not too pronounced on well-dialyzed samples and for the most part the schlieren patterns appeared enantiographic. The pH values of the buffers were measured at 3° with a Radiometer pHM 22p meter, equipped with a scale expander type pHA and A. H. Thomas glass-calomel electrodes.

Partial Specific Volumes. Apparent partial specific volumes were determined pycnometrically. The pycnometers were of the self-adjusting capillary type blown by Microchemical Specialties Co. according to the design of Anderson (1948), with slight modifications (Yue *et al.*, 1967). The volume of the pycnometer was calibrated with water and was found to be 0.96481 ml at 3° and 0.96506 ($\pm 8 \times 10^{-6}$) ml at 20.0°. Experimental details for weighing the temperature-equilibrated pycnometer have been described (Yue *et al.*, 1967). The apparent partial specific volume was calculated according to Kraemer (1940).

Other Methods. Weight fractions of the protein solutions, which were required for the extinction coefficient and biuret factors, were determined by drying aliquots (at approximately 74°) in tared microplatinum boats to constant weight in an evacuated (0.05 mm) pistol tube containing P_2O_5. Weighings were carried out to the nearest 0.001 mg. Ash was determined on these dried samples in the platinum boats by igniting in a double porcelain crucible over a meker burner. Aliquots of the dialysis fluids were subjected to the identical treatment (and at the same time) and the final dried weights (and ash) of the protein sample were determined by difference.

Measurements of the ultraviolet absorption spectra and biuret values (Gornall, 1949) were made on aliquots of the same protein solutions whose weight fractions were determined. This permitted a calculation of the extinction coefficients and biuret factors, which were then employed as secondary measures of the dry weight of the protein. Spectral measurements were made with a Beckman DU spectrophotometer. Values for the extinction coefficients reported here were made in 0.05 M sodium phosphate (pH 7.00) at 25°.

Results and Discussion

Sedimentation Velocity. Because of the large dependency of its apparent isoelectric point on ionic strength (to be described below), the sedimentation velocity behavior was explored over the pH region of 5.6–7.9, at ionic strengths of *ca.* 0.15–0.17. Typical velocity patterns for initial protein concentration of *ca.* 6–7 mg/ml are shown in Figure 2, where the protein appears to sediment as a single species.

At relatively high protein concentrations (*ca.* 10–12 mg/ml, and especially in 0.15 M KCl–0.010 M phosphate buffer, pH 6.8), although only a single well-defined symmetrical schlieren boundary is observable, careful examination of the base line of the schlieren pattern on

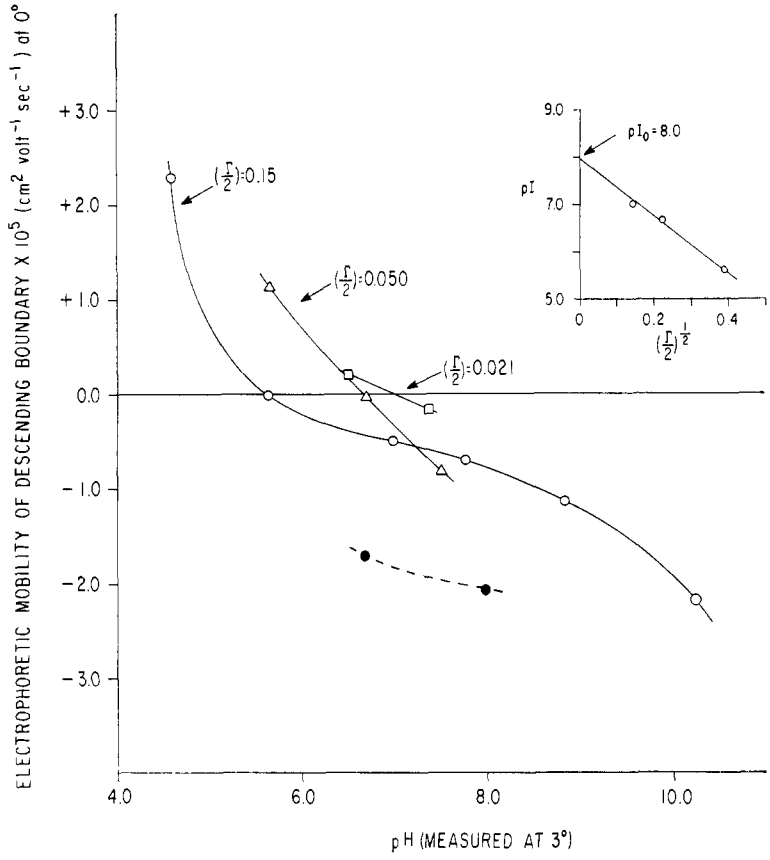

FIGURE 5: Electrophoretic mobility of 0° of nucleoside triphosphate–nucleoside diphosphate transphosphorylase as a function of pH (measured at 3°) at several ionic strengths. Lower graph: (O——O) 0.15 ionic strength buffers containing 0.105–0.125 ionic strength KCl; buffers: glycine–KCl–HCl, Tris–KCl–HCl, imidazole–KCl–HCl, and potassium acetate–KCl–acetic acid at pH 10.25, 8.84, 7.77, 6.98, 5.61, and 4.57, respectively. (△——△) 0.050 ionic strength buffers; buffer: Tris–HCl, imidazole–HCl, and potassium acetate–acetic acid at pH 7.51, 6.69, and 5.64, respectively. (□——□) 0.021 ionic strength buffers; buffer: imidazole–HCl at pH 7.37 and 6.49. (●---●) 0.15 ionic strength phosphate–KCl buffers containing 0.050 M total phosphate as pH 7.98 and 6.88. Upper graph: p*I*, isoelectric point, *vs.* $(\Gamma/2)^{1/2}$, square root of the ionic strength.

the trailing side of the peak shows what appears to be a minute elevation in the base line and which may be indicative of an interacting system (see below), since it disappears on dilution. The observed sedimentation coefficients (at 3° and buffer) appear to decrease with an increase in protein concentration (Figure 3), according to the expressions (where $\bar{c}$ is expressed in terms of the average boundary concentration in milligrams per milliliter): at pH 5.6 ($\Gamma/2$ = 0.15, KCl–acetate), $s'_{3^\circ,b}$ = $(3.85 - 0.035\bar{c})10^{-13}$ sec; and at pH 7.9 (0.15 M KCl–0.01 M Tris), $s'_{3^\circ,b}$ = $(3.87 - 0.033\bar{c}) \times 10^{-13}$ sec. Under these two conditions, the concentration dependency is not unusual (*cf.* the case of glucose 6-phosphate dehydrogenase; Yue *et al.*, 1967). However, in 0.01 M phosphate–0.15 M KCl (pH 6.8) the nonlinear concentration dependency required the use of a two-term expression to adequately account for all the data and to permit an accurate extrapolation to zero protein concentration, *viz.*, $s'_{c^\circ,b}$ = $(3.76 - 0.0028c - 0.0037\bar{c}^2) \times 10^{-13}$ sec.

Extrapolation to zero protein concentration yields values of $s^0_{3^\circ,b}$ = 3.85 and 3.87 S at pH 5.6 and 7.9, respectively, and 3.76 S at pH 6.8; or after correction to the reference states of 20° and water, of 5.67, 5.82, and 5.58 (for pH 5.6, 7.9, and 6.8, respectively), with little significant differences between these values. Thus, although some evidence for nonideality effects appears to manifest itself in dilute phosphate buffers (0.15 M in KCl), there does not appear to be any unusual effect of pH between 5.6 and 7.9 (the former pH value being close to its apparent isoelectric point at this ionic strength, see below); but the small negative slopes at pH 5.6 and 7.9 would not preclude weak interactions (Nichol *et al.*, 1964).

Free Diffusion Studies and Molecular Weight by Sedimentation Velocity and Diffusion ($M_{s,D}$). Measurements of the translational diffusion coefficients were measured under identical conditions of temperature, buffer species, and ionic strength as that employed for the sedimentation velocity studies at pH 5.6 and 7.9. The protein appeared to diffuse as a single component, under each set

of conditions, and with no evidence of other protein components with widely differing diffusion coefficients, yet the concentration dependencies appeared somewhat abnormal. These data are summarized in Figure 1, in a plot of the diffusion coefficient *vs.* J (number of Rayleigh fringes) and may be fitted to the linear statistical relationships: $D_{3^\circ,b} = (3.34 + 0.0029J) \times 10^{-7}$ cm²/sec for pH 7.9, and $D_{3^\circ,b} = (3.35 + 0.0033J) \times 10^{-7}$ cm²/sec for pH 5.6. Thus, only very slight *positive* slopes are observable in both cases, for $D_{3^\circ,b}$ *vs.* concentration, and when coupled with the sedimentation velocity data (Figure 3) it would imply a dependency of the calculated molecular weight with protein concentration, if the classical Svedberg equation ($M_{s,D} = RTS/[D(1 - \bar{V}\rho)]$) were employed without correction for nonideality. That is, a significant departure from ideality would be evident at high protein concentrations, if the expression $M_{s,D} = \{RTS/[D(1 - \bar{V}\rho)]\}\{1 + (\mathrm{d}(\ln\gamma)/\mathrm{d}(\ln c))\}$ were appropriate, and if the thermodynamic term in the brackets were ignored (where γ is the activity coefficient and c the concentration of protein; *e.g.*, see Schachman, 1959). Extrapolation to zero protein concentration should reduce the thermodynamic correction to zero, and values of $D_{3^\circ,b}$ of 3.34 and 3.35 $\times 10^{-7}$ cm²/sec at pH 7.9 and 5.6 are obtained at infinite dilution. After correction to the reference states of 20° and water, extrapolated values of $D^0_{20,w}$ of 5.69 and 5.63 $\times 10^{-7}$ cm²/sec, respectively, are calculated and which are in excellent agreement with each other. (Coincidentally, the refractive index increment at 546 mμ and 3° was determined under both of these conditions, *viz.*, 1.93 $\times 10^{-3}$ (g/100 ml)$^{-1}$). Application then of the *limiting expression* for sedimentation velocity and diffusion, together with measured values for the apparent partial specific volume of the protein, gives a calculated value of the apparent weight-average molecular weight of 102,200 (with an estimated uncertainty of ±2000), at pH 5.6, and 103,700 (±3000) at pH 7.9. Within their uncertainties (largely owing to inherent errors in extrapolation) both values are identical, but the former value would appear to be somewhat more reliable.

Sedimentation Equilibrium Studies and Molecular Weight. Since an unusual departure from ideality at high protein concentrations was evident, confirmation of the kinetic unit molecular weight (of *ca.* 102,000, *vide supra*) was sought by true sedimentation equilibrium analysis, and a typical analysis (at pH 5.9, for an initial protein concentration of *ca.* 5 mg/ml) is presented in Figure 4, in terms of a plot of log J *vs.* x^2 at sedimentation equilibrium. Within the deviations at the extreme ends of the cell (see the lower "deviation plot," Figure 4) where significant errors in measurement are evident, the relatively low ratio calculated for the z-average molecular weight to weight-average molecular weight (1.03) would be evidence of homogeneity.

The value of the weight-average molecular weight throughout the entire contents of the cell ($\bar{M}_w$) was calculated to be *ca.* 95,700, and in approximate agreement with a calculated value of $M_{s,D}$ for this finite protein concentration, but slightly lower than the extrapolated value of $M^0_{s,D}$ at zero protein concentration. Therefore,

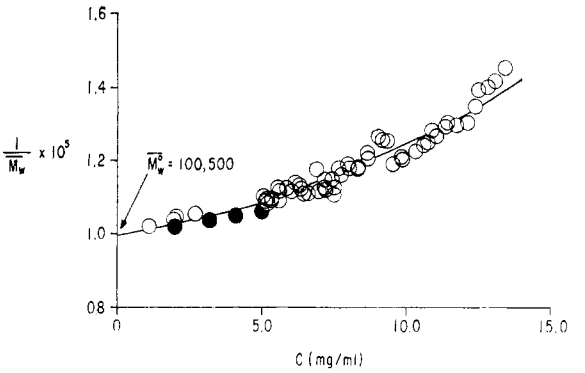

FIGURE 6: Apparent weight-average molecular weight of nucleoside triphosphate–nucleoside diphosphate transphosphorylase by Ehrenberg's "approach to sedimentation equilibrium" method in 0.141 M KCl–0.009 M potassium acetate, (Γ/2) = 0.15, pH 5.6, 3°, and 12,590 rpm. The filled circles represent the apparent weight-average molecular weight of nucleoside triphosphate–nucleoside diphosphate transphosphorylase in 0.0884 M KCl–0.0092 M KH_2PO_4–0.0008 M K_2HPO_4 (pH 5.9), (Γ/2) = 0.100 at 3°, by the true sedimentation analyses for initial protein concentrations of 2.0, 3.2, 4.1, and 5.0 mg/ml. The statistical equation for the entire plot is $1/M_{w\ app} = (0.995 + 0.010c + 9.0015c^2) \times 10^{-5}$, where c represents the meniscus concentrations (in milligrams per milliliter) for the Ehrenberg runs (○—○) and the initial concentration for the true sedimentation equilibrium analyses (●——●) $\bar{M}^\circ_w = 100{,}500 \pm 4000$.

values for $\bar{M}_w$ were determined at several initial protein concentrations by true sedimentation equilibrium analyses; and, in addition, in order to explore the concentration dependency of M_w at relatively high protein concentrations, the Ehrenberg adaptation of Archibald's approach to equilibrium method (see Experimental Section) was employed. These values are all summarized in Figure 6, in terms of a plot of the reciprocal of the apparent weight-average molecular weight *vs.* the meniscus concentration. As may be seen, the true sedimentation equilibrium analyses also fit nicely to the over-all plot for the Ehrenberg runs at the relatively low concentration range where the former technique is applicable and in which region the concentration dependency is the least. At relatively high values of protein concentration a marked concave upward plot is evident, and the entire set of data fit the two-term statistical equation, $1/\bar{M}_{w\ app} = (0.995 + 0.010c + 0.0015c^2) \times 10^{-5}$, which permits a more reliable extrapolation of $\bar{M}_w$ to zero protein concentration (than by a simple M_{app} *vs.* c plot), *viz.*, $\bar{M}^0_w = 100{,}500$ (with an estimated uncertainty of ±4000). But within the uncertainties of the extrapolated values, the estimates for the molecular weights by sedimentation velocity and diffusion and by sedimentation equilibrium are in agreement; and the most reliable estimate for $\bar{M}^0_w$ is taken to be 102,000 (see above).

For an ideal protein solute or at zero protein concentration, the expression $M_{w,x} = \{2RT/[(1 - \bar{V}\rho)\omega^2]\} \cdot \{d \ln c_x/d(x^2)\}$ would be assumed to hold at sedimentation equilibrium; however, for a real solute, at finite protein concentration, the sedimentation equilibrium expression should be amended to $M_{w,x} = \{2RT/[(1 - \bar{V}\rho)\omega^2]\}\{d \ln c/d(x^2)\}(1 + d \ln \gamma/d \ln c)$, where $M_{w,x}$ is interpreted as the weight-average molecular weight at the solute concentration c and radial position x, and γ is the activity coefficient (Goldberg, 1953; Schachman, 1959). Since the value of $(d \ln \gamma/d \ln c)$ is not known, one approach defines $\ln \gamma$ by a suitable power series in concentration (Goldberg, 1953; Mandelkern *et al.*, 1957; Wales *et al.*, 1951; Williams *et al.*, 1958; Fujita, 1962). Thus, rearranging, $[(1 - \bar{V}\rho)\omega^2/(2RT)][d(x^2)/d \ln c] = (1/M) + 2B_2c + 3B_3c^2 + \ldots$, where the subscripts w and x have been omitted, and B_2, B_3, etc., are the virial coefficients analogous to the nonidealty factors in osmotic pressure data (Wales, 1948; Kielley and Harrington, 1960). At the meniscus in the Archibald runs or at any radial position in the true sedimentation equilibrium runs, one may define $1/\bar{M}_{w\,app} = (1/M) + 2B_2c + 3B_3c^2 + \ldots$ (Wales, 1948; Kielley and Harrington, 1960). Thus, the statistical relation fitting the experimental data, $1/\bar{M}_{w\,app} = (0.995 + 0.010c + 0.0015c^2) \times 10^{-5}$, yields the second virial coefficient B_2 from the limiting slope (1.0×10^{-7}, where c is also in grams per liter); *i.e.*, $B_2 = (1.0/2) \times 10^{-7} = 5 \times 10^{-8}$ mole l./g^2 or 5×10^{-5} mole ml/g^2, if c is expressed in g/ml as appears to be the convention (Kielley and Harrington, 1960; Tanford, 1961). The case of myosin seems an apt analogy for the present situation and a comparison of this value of B_2 with that determined by Kielley and Harrington (1960) or by Mueller (1964) for myosin, which also displays a positive second virial coefficient of 9 or 10×10^{-5} mole ml/g^2, respectively, shows that the nucleoside diphosphokinase displays a much smaller concentration dependency than the rodlike myosin molecule (Kielley and Harrington, 1960). Apparently, the effects of thermodynamic nonidealty, *i.e.*, interactions (or possibly association–dissociation in a rapidly adjusting equilibria), are manifested to a much larger degree in the myosin case than in the present situation, but these effects may not be ignored in the present case if any adequate estimation of its moelcular weight is to be made. Tanford (1961) has tabulated values of B_2M for several proteins (from osmotic pressure measurements), and a value of $B_2M = 5.0$ cm^3/g may be estimated for the nucleoside diphosphokinase, which can be compared with his tabulated values of 3.2 for β-lactaglobulin, 3.7 for hemoglobin, and 75 for myosin (actually, 54–56 for myosin, if one uses the sedimentation data of Mueller (1964) or of Kielley and Harrington (1960)). Thus, the nucleoside diphosphokinase has a B_2M value somewhat higher than the theoretical value of 3.0 for a spherical molecule (*e.g.*, see Tanford, 1961), but far less than the theoretical value of 75 for an equivalent rodlike molecule of length/diameter of 100. The f/f_0 value, estimated for this protein from its extrapolated coefficients corrected to 20° and water, is only *ca.* 1.19, with a calculated axial ratio for an assumed anhydrous prolate ellipsoid of revolution of only *ca.* 4.2 (Perrin, 1936). It may be estimated (*e.g.*, see Edsall, 1953) that if the protein imbibed a significant amount of tightly bound solvent (w) to the extent of 0.5 g/g of protein, then $V_{\text{mole hydrated}} \cong M\bar{V}[1 + (w/\bar{V}\rho_0)] = 126$ l./mole, and which would yield a $(f/f_0)_{\text{hydration}} \cong [1 + (w/\bar{V}\rho)]^{1/3} = 1.18$, or $(f/f_0)_{\text{asym}} = (f/f_0)/(f/f_0)_{\text{hydration}} = 1.19/1.18 \cong 1.01$, a value essentially that of a swollen or expanded sphere (Oncley, 1941). The value of $V_{\text{mole hydrated}}$ may then be compared with that which may be estimated from its value of B_2, since for a sphere the excluded volume $\cong 4 \times V_{\text{mole}} \cong B_2M^2$ or $V_{\text{mole}} \cong 126$ l./mole and which is the same value estimated for a hydrated sphere 0.5 g/g of hydration. The radius of a hydrated sphere of $V_{\text{mole}} = 126$ l./mole may be computed next from $r_{\text{hydrated}} = [3V_{\text{hydrated}}/(4\pi N)]^{1/3} \cong 37$ A. Thus, this value for the radius of a hypothetical hydrated and expanded sphere might, in part, be reflected in the slightly higher value of B_2M compared to a few other globular proteins; and this protein, as a result of imbibition of tightly bound solvent to yield perhaps a model approaching that of a swollen sphere, might resemble the case of the expanded albumin molecule under acidic conditions (*e.g.*, see Tanford, 1961).

It is pertinent perhaps at this point to indicate that a few preliminary data were obtained also at pH 7.9, as a function of protein concentration. Although the extrapolated value of $\bar{M}^0_{\text{app}}$ was approximately the same *viz.*, $(100\text{–}104) \times 10^3$, nevertheless, the limiting slope (of a $1/\bar{M}_{\text{app}}$ *vs.* c_m plot) appeared to approximately double, indicative of the effect of charge on the second virial coefficient, in a manner similar to that described by Scatchard *et al.* (1946) and by Edsall *et al.* (1950) for the effect of charge on the second virial coefficient of serum albumin. At present, therefore, it is assumed that the second virial coefficient at pH 5.6–5.9 ($\Gamma/2 \cong 0.15$), near its apparent isoelectric point for this ($\Gamma/2$), would include a negligible Donnan term; and that the primary concentration effect is due to departure from thermodynamic idealty, *i.e.*, an effect on the activity coefficients, or possibly an alteration in the frictional coefficient with concentration in the velocity and diffusion experiments, as a result of preferential binding of solvent components. It would be tempting at this point to also speculate on the possibility of an association–dissociation set of equilibria (a preliminary amino acid analysis would point to the existence of an n-mer); however, since further studies are in progress which are designed to shed more direct light on this particular point, this particular discussion is more appropriately postponed to a later communication, except for the following comment. An estimate of the third virial coefficient in the relatively high protein concentration range is subject to a very large uncertainty. However, it appears qualitatively to be larger than is to be expected according to any solution theory (*cf.* Hill, 1960) and possibly is a reflection of traces of light material (see above under sedimentation velocity) which are overweighted in the analyses at the meniscus by the Ehrenberg method; again a complex association–dissociation equilibria is a likely contributor to the high protein concentration region.

Liquid-Boundary Electrophoresis Studies. The liquid-boundary electrophoretic behavior of the protein (at 1°) was examined over a range of pH values (from *ca.* 4.6 to *ca.* 10.3 at $(\Gamma/2) = 0.15$) at several ionic strengths (from 0.021 to 0.15) and in the presence of a number of buffer species. A summary of the data obtained is presented in Figure 5, in terms of several plots of mobility *vs.* pH at constant ionic strength. The isoelectric states (defined as the points of zero mobility) were a dramatic function of the ionic strength; and a plot of *pI vs.* $(\Gamma/2)^{1/2}$ (upper right-hand insert of Figure 5) permitted a linear extrapolation to zero ionic strength, *i.e.*, an extrapolated value of $pI_0 \cong 8.0$ at $(\Gamma/2 = 0)$ is obtained. There is almost a change of 2.4 pH units in the value of *pI*, from $(\Gamma/2) \cong 0.15$ ($pI \cong 5.6$) to zero ionic strength indicative of unusual charge interactions or a change in frictional coefficient with ionic strength as a result of preferential binding of solvent components. That the observed electrophoretic mobilities are influenced by the buffer species to some extent, is evidenced by the marked change in mobilities (*i.e.*, negative) in phosphate–KCl buffers (Figure 6) compared to imidazole–HCl–KCl buffers, at the same total ionic strength, which might also imply a strong interaction with the phosphate buffer species (*cf.* similar results by Yue *et al.* (1967) on glucose 6-phosphate dehydrogenase and Barlow and Margoliash (1966) on several cytochrome's *c*). In view of the relative lack of specificity this enzyme displays towards its nucleotide substrates (Chiga and Plaut, 1962; Chiga *et al.*, 1963; Ratliff *et al.*, 1964; Nakamura and Sugino, 1966; Mourad and Parks, 1966; Goffeau *et al.*, 1967), as well as the implicated phosphoryl enzyme as a catalytic intermediate (Mourad and Parks, 1965; Norman *et al.*, 1965), it is perhaps not too surprising that the enzyme would interact strongly with the phosphate species.

It will be of interest to correlate the marked effect of ionic strength on *pI*, and its extrapolated pI_0, with its amino acid composition and titrimetric studies, which in turn might reveal what charge interactions are responsible for this dramatic effect. Parenthetically, it may be remarked that aldolase appeared to yield similar electrophoretic behavior as well as interactions with the phosphate buffer species (Velick, 1949), and in view of the subunit structure revealed for aldolase (Stellwagen and Schachman, 1962; Kawahara and Tanford, 1966; Deal *et al.*, 1963; Chan and Morse, 1967), interactions between hypothetical subunits as a function of ionic strength as well as preferential binding might be contributing factors in the electrophoretic behavior.

In Table I are summarized all the physical properties pertinent to this report, and which also include its extinction coefficients at 282 mμ (max) and a ratio of values at 282:259 mμ, the biuret factor refractive index increment for 3° and 546 mμ, and values for the apparent partial specific volume measured at 3 and 20°.

By the several physical techniques employed for these studies (sedimentation velocity, sedimentation equilibrium, diffusion, and electrophoresis) no evidence for any gross heterogeneity could be obtained; however, implications of departure from thermodynamic ideality were evident and have been described above. The nature of the interactions responsible are not entirely clear, but at pH values far removed from its apparent isoelectric state charge effects (see under sedimentation equilibrium), interactions with buffer species (see electrophoretic studies) are distinct possibilities. At pH values near its apparent isoelectric point, effects on the frictional coefficient by preferential binding of solvent components, the effects of protein concentration on the activity coefficients, and repulsive forces between possible subunits, all combine to yield a positive second virial coefficient. A complex and rapidly adjusting set of association–dissociation equilibria may also contribute and this effect is the subject of current investigations. It is possible that the over-all kinetic molecular unit (and whose best value of the molecular weight appears to be 102,000) might approximate a swollen or expanded

TABLE I: Physical Properties of Nucleoside Triphosphate–Nucleoside Diphosphate Transphosphorylase.

pI_0, isoelectric point $(\Gamma/2 \rightarrow 0)$	8.0
$s^0_{3°,b}$, sedimentation coefficient ($c \rightarrow 0$, pH 5.6) (sec)	3.85×10^{-13}
$s^0_{20,w}$, sedimentation coefficient ($c \rightarrow 0$, pH 5.6) (sec)	5.67×10^{-13}
$D_{3°,b}$, diffusion coefficient ($c \rightarrow 0$, pH 5.6) (cm^2/sec)	3.35×10^{-7}
$D_{20,w}$, diffusion coefficient ($c \rightarrow 0$, pH 5.6) (cm^2/sec)	5.63×10^{-7}
$M_{s,D}$, molecular weight by sedimentation and diffusion ($c \rightarrow 0$) (g/mole), pH 5.6 (pH 7.9 = 103,700 ± 3000)	102,000 ± 2000
M_{equil}, molecular weight by sedimentation equilibrium (g/mole) ($c \rightarrow 0$), pH 5.9	100,500 ± 4000
$\bar{V}_{app,3°}$, apparent partial specific volume ($c = 1.8\%$) (cm^3/g)	0.736
$\bar{V}_{app,20°}$, apparent partial specific volume ($c = 1.8\%$) (cm^3/g)	0.761
f/f_0, molar frictional ratio	1.19 ($a/b = 4.2$ for anhydrous prolate)
B_2, second virial coefficient (mole ml/g^2)	5.0×10^{-5}
$(\Delta n/\Delta c)_{3°}$, refractive index increment (546 mμ) (g/100 ml)$^{-1}$	1.93×10^{-3}
Biuret factor (540 mμ, 10-ml volume, 1.0-cm light path) (mg/absorbance unit)	34.2
$E^{1\%}_{1\ cm}$, extinction coefficient (282 mμ, pH 7.00, 0.05 M phosphate)	16.04
Ratio of extinction coefficients at 282:259 mμ	2.01

sphere, under the conditions of measurement. Future investigations by other techniques more specifically designed to unravel its three-dimensional structure are areas for later studies.

Acknowledgments

The authors wish to thank Anheuser-Busch, Inc. (Dr. R. Seeley and M. Dacksel), for generous quantities of their dried brewers' yeast. The technical assistance of Mr. C. Richardson is gratefully acknowledged.

References

Abramson, H. A., Moyer, L. S., and Gorin, M. H. (1942), Electrophoresis of Proteins, New York, N. Y., Reinhold.

Alberty, R. A. (1954), *J. Am. Chem. Soc. 76*, 3733.

Anderson, H. H. (1948), *Anal. Chem. 20*, 1241.

Archibald, W. J. (1947), *J. Phys. Colloid Chem. 51*, 1204.

Baldwin, R. L. (1957), *Biochem. J. 65*, 503.

Barlow, G. H., and Margoliash, E. (1966), *J. Biol. Chem. 241*, 1473.

Chan, W., and Morse, D. (1967), *Federation Proc. 26*, 602.

Chiga, M., Oda, A., and Holtzer, R. L. (1963), *Arch. Biochem. Biophys. 103*, 366.

Chiga, M., and Plaut, G. W. E. (1962), *Biochim. Biophys. Acta 61*, 736.

Deal, W. C., Rutter, W. J., and Van Holde, K. E. (1963), *Biochemistry 2*, 246.

Edsall, J. T. (1953), *Proteins 1*, 549.

Edsall, J. T., Edelhoch, H., Lontie, R., and Monson, P. R. (1950), *J. Am. Chem. Soc. 72*, 4641.

Ehrenberg, A. (1957), *Acta Chem. Scand. 11*, 1257.

Fujita, H. (1962), Mathematical Theory of Sedimentation Analysis, New York, N. Y., Academic.

Goffeau, A., Pedersen, P. L., and Lehninger, A. L. (1967), *J. Biol. Chem. 242*, 1845.

Goldberg, R. J. (1953), *J. Phys. Chem. 57*, 194.

Gornall, A. G. (1949), *J. Biol. Chem. 177*, 751.

Gosting, L. J. (1956), *Advan. Protein Chem. 11*, 430.

Gosting, L. J., and Morris, M. S. (1949), *J. Am. Chem. Soc. 71*, 1998.

Gropper, L. (1964), *Anal. Biochem. 7*, 401.

Hexner, P. E., Radford, L. E., and Beams, J. W. (1961), *Proc. Natl. Acad. Sci.. U. S. 47*, 1848.

Hill, T. (1960), Introduction to Statistical Thermodynamics, New York, N. Y., Addison-Wesley.

Jullander, I. (1945), *Arkiv. Mineral. Geol. 21A*, 1.

Kawahara, K., and Tanford, C. (1966), *Biochemistry 5*, 1578.

Kegeles, G., and Gutter, F. J. (1951), *J. Am. Chem. Soc. 73*, 3770.

Kegeles, G., Klainer, S. M., and Salem, W. J. (1957), *J. Phys. Chem. 61*, 1286.

Kielley, W. W., and Harrington, W. F. (1960), *Biochim. Biophys. Acta 41*, 401.

Kraemer, E. O. (1940), *in* The Ultracentrifuge, Svedberg, T., and Pedersen, K. O., Ed., London, Oxford University, p 57.

Kuby, S. A., Noda, L., Lardy, H. A. (1954), *J. Biol. Chem. 209*, 191.

LaBar, F. E. (1966), *Biochemistry 5*, 2368.

Longsworth, L. G. (1942), *Chem. Rev. 30*, 323.

Longsworth, L. G. (1947), *J. Am. Chem. Soc. 69*, 2510.

Longsworth, L. G. (1952), *J. Am. Chem. Soc. 74*, 4155.

Mandelkern, L., Williams, L. C., and Weissberg, S. G. (1957), *J. Phys. Chem. 61*, 271.

Mourad, N., and Parks, R. E., Jr. (1965), *Biochem. Biophys. Res. Commun. 19*, 312.

Mourad, N., and Parks, R. E., Jr. (1966), *J. Biol. Chem. 241*, 271.

Mueller, H. (1964), *J. Biol. Chem. 239*, 797.

Nakamura, H., and Sugino, Y. (1966), *J. Biol. Chem. 241*, 4917.

Nichol, L. W., Bethune, J. L., Kegeles, G., Hess, E. L. (1964), *Proteins 2*, 305.

Noda, L., and Kuby, S. A. (1957), *J. Biol. Chem. 226*, 541.

Norman, A. W., Wedding, R. T., and Black, M. K. (1965), *Biochem. Biophys. Res. Commun. 20*, 703.

Oncley, J. L. (1941), *Ann. N. Y. Acad. Sci. 41*, 121.

Perrin, F. (1936), *J. Phys. Radium 7*, 1.

Ratliff, R. L., Weaver, R. H., Lardy, H. A., and Kuby, S. A. (1964), *J. Biol. Chem. 239*, 301.

Richards, E. G., and Schachman, H. K. (1959), *J. Phys. Chem. 63*, 1578.

Scatchard, G., Batchelder, A. C., and Brown, A. (1946), *J. Am. Chem. Soc. 68*, 2320.

Schachman, H. K. (1957), *Methods Enzymol. 4*, 32.

Schachman, H. K. (1959), Ultracentrifugation in Biochemistry, New York, N. Y., Academic, pp 222 ff, 77, and 203.

Spinco Division, Beckman Instruments, Inc. (1964), Technical Manual No. E-IM-3.

Stellwagen, E., and Schachman, H. K. (1962), *Biochemistry 1*, 1056.

Svedberg, T., and Pedersen, K. (1940), The Ultracentrifuge, London, Oxford University.

Tanford, C. (1961), Physical Chemistry of Macromolecules, New York, N. Y., Wiley.

Tiselius, A. (1937), *Trans. Faraday Soc. 33*, 524.

Trautman, R., Schumaker, V. N., Harrington, W. F., and Schachman, H. K. (1954), *J. Chem. Phys. 22*, 555.

Van Holde, K. E., and Baldwin, R. L. (1958), *J. Phys. Chem. 62*, 734.

Velick, S. F. (1949), *J. Phys. Colloid Chem. 53*, 135.

Wales, M. (1948), *J. Phys. Colloid Chem. 52*, 235.

Wales, M., Adler, F. T., and Van Holde, K. E. (1951), *J. Phys. Colloid Chem. 55*, 145.

Weaver, R. H. (1962), *Enzymes 6*, 151.

Williams, J. W., Van Holde, K. E., Baldwin, R. L., and Fujita, H. (1958), *Chem. Rev. 58*, 715.

Yphantis, D. A. (1964), *Biochemistry 3*, 297.

Yue, R. H., Noltmann, E. A., and Kuby, S. A. (1967), *Biochemistry 6*, 1174.

Yue, R. H., Ratliff, R. L., and Kuby, S. A. (1965), *Federation Proc. 24*, 284.