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LIGHT-SCATTERING STUDIES OF THE α-KETOGLUTARATE DEHYDROGENASE COMPLEX FROM *ESCHERICHIA COLI*

I. CHARACTERIZATION OF THE SELF-ASSOCIATION OF THE COMPLEX

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The self-association of *Escherichia coli* α-ketoglutarate dehydrogenase complex (KGDC) purified by a column chromatographic technique, was characterized by light-scattering photometry. The complex adopts a solution conformation somewhat larger than that observed in the electron microscope. The evidence suggests a nonideal indefinite self-association model for KGDC in KCl, phosphate buffer. The KGDC monomer has a molecular charge of about -3×10^2 at neutral pH. The self-association is promoted by increasing KCl concentrations, pH (in the range from 6.3 to 7.4) and temperature (from 20 to 30°C). The effects of pH changes suggest a release of protons during the self-association and a minor 'preferential' interaction of phosphate ions. For the association of one monomer to the aggregate at neutral pH and 25°C, $\Delta G^\circ = -7.8$ kcal mol^{-1}, $\Delta H^\circ = 24$ kcal mol^{-1} and $\Delta S^\circ = 1.1\times10^2$ cal mol^{-1} K^{-1}. These data indicate that hydrophobic interactions drive the association. Thermodynamically, the self-association of KGDC is a complex phenomenon and may serve to stabilize the enzyme complex in solution.

1. Introduction

The α-ketoglutarate dehydrogenase multienzyme complex (KGDC) catalyzes the reaction of α-ketoglutarate, NAD^+ and coenzyme A to yield CO_2, NADH and succinyl-coenzyme A. The enzymatic properties and architecture of the *Escherichia coli* and mammalian KGDC have been elucidated by Reed [1], and more recently by Angelides and Hammes [2]. The *E. coli* KGDC consists of three enzymes. The transuccinylase component self-associates to form an approximately cubic core containing 24 components, and to it are added 6 mol each of α-ketoglutarate dehydrogenase dimers and FAD-containing dihydrolipoyl dehydrogenase dimer to form KGDC.

In the investigation of the thermodynamics of the associations involving the three component enzymes of KGDC, we were confronted with a complex system which involves many equilibria. Our approach has been to characterize systematically the interactions involved in the equilibria between the individual components with the hope of achieving therefrom an understanding of the entire system. We present herein a thermodynamic analysis of the nonideal self-association of *E. coli* KGDC based on light-scattering measurements.

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Abbreviations: KGDC, α-ketoglutarate dehydrogenase complex; V_0, void volume of column; ^zR_G, radius of gyration; $\overline{M}^a_w$, apparent weight average molecular weight; K_D, association constant for dimerization; K_I, association constant for indefinite self-association.

2. Materials and methods

2.1. KGDC isolation and purification

The enzyme was isolated from Crooks strain *E. coli* (American Type Culture Collection) aerobically cultured to 8 g cells/l in the medium described by Reed and Mukherjee [3] supplemented with 4% glutamic acid [4] and 3% ethanol. The procedure used to isolate large quantities of KGDC from the sonicated cells partially followed that of Reed and Mukherjee [3] and is summarized in table 1.

Because of the spontaneous degradation of 'pure' KGDC solutions [6,7], the frozen KGDC samples were thawed at 5°C, centrifuged at 5×10^4 *g* for 30 min, and chromatographed (in the desired buffer) on a Sepharose 4B column (fractions between 1.45 and $1.65V_0$ were taken) immediately prior to use. Sedimentation velocity (fig. 1) and agarose-polyacrylamide gel electrophoresis experiments indicate the KGDC preparation so obtained has less than 0.5% contaminants. KGDC used for all subsequent experiments was prepared by this procedure.

2.2. Extinction coefficients and protein measurement

All routine protein concentration measurements were spectrophotometric. The extinction coefficients of KGDC and lysozyme were determined by the dry-weight method. While the procedure described by Edsall and Wyman [8] which requires drying the protein at its isoelectric pH was suitable for lysozyme, the precipitation of KGDC at its isoelectric point [3] required some additional steps before the dry-weight procedure could be used. A concentrated solution of KGDC was converted to its lithium salt by passage through a Bio-Gel P6 column equilibrated with 0.5 M LiCl (pH 7.0) and subsequently freed of excess LiCl by passage through a Bio-Gel P-6 column equilibrated with distilled water. Atomic absorption spectrophotometric measurement of this final solution indicated an absence of the cations used in purification (i.e., Mg^{2+}, Na^+, K^+), and the presence of 2.0 mg Li^+/g dry KGDC. Cl^- was absent as indicated by the $AgNO_3$ test.

Table 1

Purification of KGDC [a]

Step	Volume (ml)	Protein concentration (mg/ml)	Specific [b] activity	KGDC recovery (mg)	% recovery
(1) Sonicated cell-free suspension	1100	26	2.6	410	(100)
(2) Supernatant, after precipitation in 7% protamine sulfate	1500	16	2.8	373	90
(3) Supernatant, after precipitation in 21% protamine sulfate	1600	6	0	0	0
(4) Recovery from three extractions of precipitate in step 3	1000	3.8	10.9	230	56
(5) Concentrate obtained by ultrafiltration over Diaflow XM 300 membrane	150	15	18	230	56
(6) Fraction centered at $1.55V_0$ on a 3 l Sepharose 4B 200 column [c]	600	(0.7) [e]	70	220	54
(7) Second fractionation on Sepharose 4B 200 column [d]	420	0.5	160	200	49

[a] 256 g of *E. coli* cell paste were used. However, 2- and 3-times this amount were also processed successfully.

[b] The assay [5] measured $Fe(CN)_6^{3-}$ reduction units $= \frac{\mu\text{mol } Fe^{3+} \text{ reduced}/2 \text{ per h}}{\text{mg protein}}$.

[c] The KGDC was concentrated after chromatography by fractional precipitation in 242 mg/ml $(NH_4)_2SO_4$.

[d] The concentrated KGDC (by precipitation with $(NH_4)_2SO_4$) remained stable up to 3 months when kept at −80°C.

[e] This concentration is an estimate read from a column monitor.

2.3. Refractive index increment measurements

The change in refractive index due to a change in the protein concentration under conditions where the chemical potentials of all solvent components are at equilibrium with the components of the bulk buffer solution, Ψ^* [9], was determined in a thermostatically controlled, calibrated [10] Brice-Phoenix differential refractometer immediately after passage of the protein through an equilibrated, thermostatically controlled Bio-Gel P-6 column in the appropriate buffer. In contrast, Ψ, the change in refractive index due to a change in protein concentration under conditions where the molar composition of all buffer components is defined, was determined by taking a volume of protein freed of excess salt by chromatography on a Bio-Gel P-6 column, and adding an appropriate amount of concentrated buffer. The protein solution was diluted with distilled water to the proper buffer concentration for measurement in the differential refractometer. The protein concentrations of the solutions were determined by absorbance measurement.

2.4. Light-scattering measurements

The light-scattering instrument incorporated the light source (a filter selected the 5460 Å line) and mechanical components of the Brice Phoenix 2000 instrument, a solid-state electronic amplifier and integrator, and a digital readout. A 2 mm wide collimating slit illuminated the cylindrical cell with flat entrance and exit sections. Thermostatically controlled water was circulated through the cell table, cell compartment and the jacket which surrounded the cell except for a slit to allow passage of the light beams. The instrument's cylindrical cell was calibrated at nine angles (between 35 and 145°) with lysozyme under conditions [11] where its molecular weight, including 16 Cl^- as counterions [12], is 14880 [13]. Hen egg lysozyme from Miles Laboratories (Grade V) was purified by chromatography on a Sephadex G-50 column immediately prior to its use.

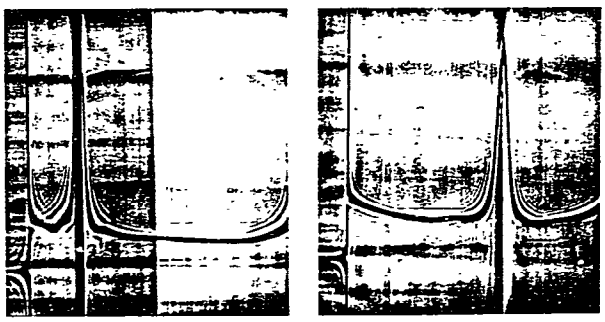

Fig. 1 Schlieren pattern of the sedimentation boundary of KGDC (10 g/l) in 0.1 ionic strength phosphate buffer, pH 7.0, 0.1 M KCl, and a temperature of 4°C. Sedimentation proceeded to the right in both the initial part of the run at an angular velocity of 4×10^4 rpm and late in the run at an angular velocity of 5.6×10^4 rpm.

The solutions used in light-scattering photometry were clarified by filtration through thoroughly washed Gelman TMC 200 or Nucleopore NO 20 filters. The cylindrical cell was washed thoroughly and rinsed repeatedly with filtered water until the intensity of scattering at 30° was reduced to a reproducible minimum value. All water was removed by a small plastic tube and the sample was introduced into the cell through the filter. A small Teflon tube allowed aspiration of the cell contents into the filter reservoir for subsequent refiltration. The cell and refiltration apparatus thus formed, in effect, a closed system. Several cycles usually yielded reproducible scattering intensities at 30°. Scattering intensity measurements were made at the nine angles for the solvent and the protein solutions. Routine light-scattering measurements were performed on progressively greater dilutions of a concentrated protein solution. Filtration of the clarified KGDC solutions was repeated until there was no change in the scattering intensities at 30°. All protein concentration measurements were performed on dilutions of the final filtered solutions following light-scattering photometry. Fluorescence and anisotropy corrections were found to be unnecessary.

Chemicals used for purification and measurement of the thermodynamic properties of KGDC were purchased from commercial sources as the highest quality available and dissolved in glass-distilled water. Protamine sulfate was purchased from Nutritional Biochemical Inc.; NAD^+ and α-ketoglutarate were purchased from Sigma.

Table 2
Physical properties of KGDC and lysozyme

Species	E [a]	Ψ^* [c]	Ψ [c]	Molecular weight [d]	R_G	Conditions [e]
KGDC	1.061 ± 0.003 [b]	473 ± 6	(468) [f]	$(2.44 \pm 0.03) \times 10^6$	14 ± 1 nm	0.1 I [h], pH 7.0, 0.1 M KCl
		479 ± 6	468 ± 5			0.1 I, pH 6.9, 0.1 M KCl, 0.05 M KI
		–	460 ± 5			0.1 I, pH 6.9, 0.1 M KCl, 0.10 M KI
		492 ± 6	465 ± 5			0.1 I, pH 6.9, 0.1 M KCl, 0.15 M KI
Lysozyme	2.613 ± 0.003	2.69 ± 0.01	–	1.488×10^4 [g]		0.5 M glycine, pH 2.5, 0.1 M NaCl

[a] The units are 1 g^{-1} cm^{-1} at a wavelength of 2770 Å for KGDC and 2800 Å for lysozyme.
[b] All uncertainties are standard errors for the sequence of measurement producing the specific result. Cumulative errors due to subsidiary measurement, e.g., in the determination of molecular weight, are not included.
[c] The units are 1 mol^{-1} at a wavelength of 5460 Å.
[d] The units are g mol^{-1}. The result for KGDC was obtained by extrapolation of the indefinite self-association equation. All measurements were made at a wavelength of 5460 Å.
[e] The phosphate buffer was prepared from monohydrogen and dihydrogen phosphate salts to a buffer ionic strength of 0.1. The temperature was 25°C.
[f] This value is the intercept of the line fitted to the other three.
[g] See the text for details on the determination of this value.
[h] I = ionic strength phosphate buffer.

3. Results

The measured physical properties of KGDC and lysozyme are shown in table 2.

3.1. Self-association of KGDC

Light-scattering photometry yields an apparent weight average molecular weight $\overline{M}_w^a$ [9,14] and a Z average radius of gyration, R_G [14] for a self-associating system. The R_G given in table 2 was obtained from the dependence of the intensity of scattered light on the observation angle (as shown in fig. 2) after extrapolation at each angle to infinite dilution. The magnitude of $\overline{M}_w^a$ at any concentration, obtained by extrapolation to an observation angle of zero, depends on the extent of self-association and departures from ideal behavior [14]. The data for KGDC shown in fig. 3 indicate the possibility of both types of behavior.

It is first necessary to identify the manner in which the KGDC self-associates. The data of fig. 3 suggest a modest association constant. Therefore, we consider the two most tractable self-association models of dimerization [15] *

$$2K_D C = \left(\frac{\overline{M}_w}{M_1} - 1\right) \Big/ \left(2 - \frac{\overline{M}_w}{M_1}\right)^2 \quad (1)$$

and indefinite self-association [16]

$$4K_I C = \left(\frac{\overline{M}_w}{M_1}\right)^2 - 1 \quad (2)$$

$\overline{M}_w$, M_1, K_D and K_I are the weight average molecular weight, the monomer molecular weight, the association constant for dimerization, and the indefinite self-association constant, respectively. The units of K are reciprocal to those chosen for the concentration, C.

Values of $\overline{M}_w$ are related to the apparent molecular weight, $\overline{M}_w^a$, by

$$\frac{1}{\overline{M}_w} = \frac{1}{\overline{M}_w^a} - 2BC \quad (3)$$

where B is the second virial coefficient. For large molecules the size of KGDC at the pH values of these measurements, the second virial coefficient is approximated by

$$B = \frac{Z_1^2}{4M_1^2 m_3} \quad (4)$$

in terms of the concentration of added salt, m_3, and the charge, Z_1, on the protein monomer [14]. For KGDC the excluded-volume effect contributes less than 20% to B and will be disregarded. At

* The term K/M_1 in eq. 7 of this reference is the K_D in eq. 1 of this paper. The difference between the two equations is due to the choice of protein concentrations units.

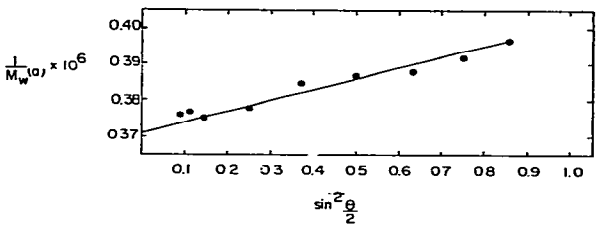

Fig. 2 A typical light-scattering photometry plot for KGDC showing the relationship between the reciprocal of the apparent molecular weight and the observation angle. The circles represent measured values at angles between 35 and 135° relative to the incident light. The line is the least-squares fit to the data points. An equivalent plot for the small protein lysozyme, would have a zero slope. The conditions are as in fig. 3; the wavelength of light was 5460 Å.

fixed pH, the charge of the monomer is assumed to be unperturbed by its association with other monomers.

To distinguish between the two models, the self-association was studied over a 10-fold range in concentrations, the greatest feasible, and under two conditions favoring the self-association to different extents. The results are given in table 3 and figs. 3 and 4.

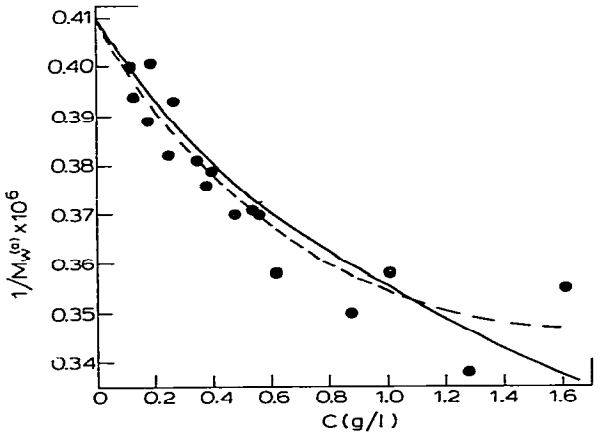

Fig. 3 The self-association of KGDC as a function of its concentration in 0.1 ionic strength phosphate buffer, pH 7.0, 0.1 M KCl, and a temperature of 25°C. The lines represent theoretical curves obtained by nonlinear regression analysis using either the dimer model (solid line) or the indefinite model (dashed line). See table 3 for the parameters of these lines.

The first group of data (A) of table 3 shows the results of nonlinear regression analysis [17] obtained when both the association constant and charge are allowed to vary [18]. The second group (B) shows the results obtained if the systems are assumed to be ideal ($B = 0$). For comparison, the third group (C) shows the results of a nonlinear regression analysis when a common monomer charge is required in both buffer systems and then the association constant is calculated for the two models.

Additional information is usually required to distinguish among modes of weak nonideal association [19]. In measuring its extinction coefficient, it was found that KGDC has 700 Li^+ as counterions per monomer at neutral pH: the maximum charge of KGDC at neutral pH in the absence of salt is -700. In view of the tendency of proteins with pI values similar to that of KGDC to bind the negative rather than the positive ions of simple salts (extensively reviewed in ref. 8, pp. 645–651) it may be reasonably inferred that KGDC bears a substantial negative charge under the conditions of our experiments. Therefore, the requirement that $Z_1 \approx 0$ (from $BM_1 \approx 0$) which follows from the dimerization model suggests that in fact the process observed is more probably indefinite self-association.

To calculate true association constants under various solvent conditions we follow the method of Paglini and Lauffer [20] who used second virial coefficients computed from excluded volume and Donnan effects to correct the apparent molecular weights at each concentration. For KGDC, the major contribution to the second virial coefficient is given by eq. 4, the excluded volume effect being small. As a practical matter, given the difficulty in ascertaining precise values of Z_1, a 'compromise' estimate of $|Z_1| = 336$, to be used at neutral pH and all ionic strengths, was obtained from the best fit of the data given in table 3, group C, in the manner described above. Values of Z_1 at other pH values were calculated on the assumption * that

* KGDC's isoelectric pH and the charge relative to mass at pH 7.0 are similar to those of bovine serum albumin. The charge of bovine serum albumin is approximately a linear function of pH in this range [8].

the charge of KGDC is a linear function of pH between the isoelectric point, pH 5.7 [3], and pH 7.0. The quantities BM_1, arrived at in this manner and the resulting estimates of equilibrium constants for indefinite self-association under various solvent conditions, derived from data illustrated in fig. 5, are given in table 4. Conclusions about the manner in which the various solvent components affect the self-association of KGDC are given below.

Table 3

Computer analysis of the self-association of KGDC under different conditions

Solvent conditions	Indefinite self-association			Dimerization		
	Association [a] constant (K_I) (l/g)	Nonideal term ($2BM_1$) (l/g)	Standard [b] error of fit (%)	Association [a] constant (K_D) (l/g)	Nonideal term ($2BM_1$) (l/g)	Standard [b] error of fit (%)
(A) Values for the best fit when the association constant and nonideal term can both vary [c]						
0.1 ionic strength [d] phosphate, pH 7, 0.1 M KCl, 25°C	0.19	0.11	1.6	0.11	0.0014	1.9
0.1 ionic strength [e] phosphate, pH 7.1, 0.5 M KCl, 25°C	0.45	0.12	0.8	0.51	−0.0011 [f]	1.4
(B) Values for the best fit when ideal behavior is assumed ($2BM_1 = 0$) [g]						
0.1 ionic strength phosphate, pH 7.0, 0.1 M KCl, 25°C	0.06		2.0	0.11		1.9
0.1 ionic strength phosphate, pH 7.1, 0.5 M KCl, 25°C	0.19		1.9	0.55		1.9
(C) Values for the best fit when the charge of the KGDC complex in the two solvent systems is required to be the same [h]						
0.1 ionic strength phosphate, pH 7.0, 0.1 M KCl, 25°C	0.20 [h]	0.12 [h]	1.6	0.48 [j]	0.12 [j]	4.9
0.1 ionic strength phosphate, pH 7.1, 0.5 M KCl, 25°C	0.31 [h]	0.039 [h]	1.8	0.72 [j]	0.039 [j]	3.3

[a] Multiply these values by M_1 to obtain the molar association constants [15].

[b] The standard error in the computed fit was defined as:

$$100\left(\frac{\Sigma\left|\text{actual }\frac{1}{M_w}-\text{calculated }\frac{1}{M_w}\right|^2}{\frac{\text{number of measurements}-1}{1/M_1}}\right)^{1/2}$$

[c] This represents the best fit to the data with K_I the association constant from eq. 2 and K_D the association constant from eq. 1.

[d] These data are shown in fig. 3.

[e] These data are shown in fig. 4.

[f] A negative second virial coefficient has no physical significance and the one given is indistinguishable from zero in the present case.

[g] These results are obtained by neglect of nonideal effects ($B = 0$) and they are presented for comparison. The indefinite model with $B = 0$ brings on a simultaneous deterioration of the fitting at both high and low protein concentrations.

[h] These results were obtained by combining the data for the two solvent conditions, allowing a different K_I under the two conditions but requiring a single value under both conditions for the adjustable parameter Z_1, the molecular charge, in finding the best fit.

[j] This is the best fit provided Z_1 is fixed at 336, the value estimated in the fit of the indefinite association model for both solvent conditions. B is calculated by eq. 4. The data at low protein concentration are very poorly represented by the dimer model under these conditions.

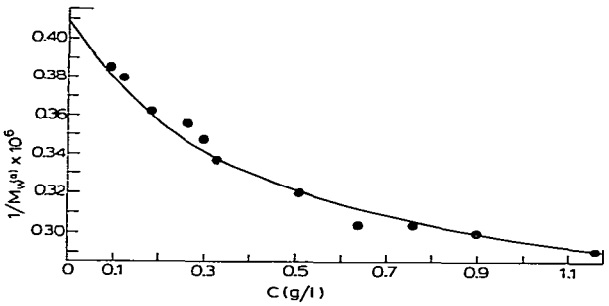

Fig. 4 The self-association of KGDC as a function of its concentration in 0.1 ionic strength phosphate buffer, pH 7.1, 0.5 M KCl, and a temperature of 25°C. The nonlinear regression analysis using the two models yields indistinguishable lines. See table 3 for the parameters of these lines.

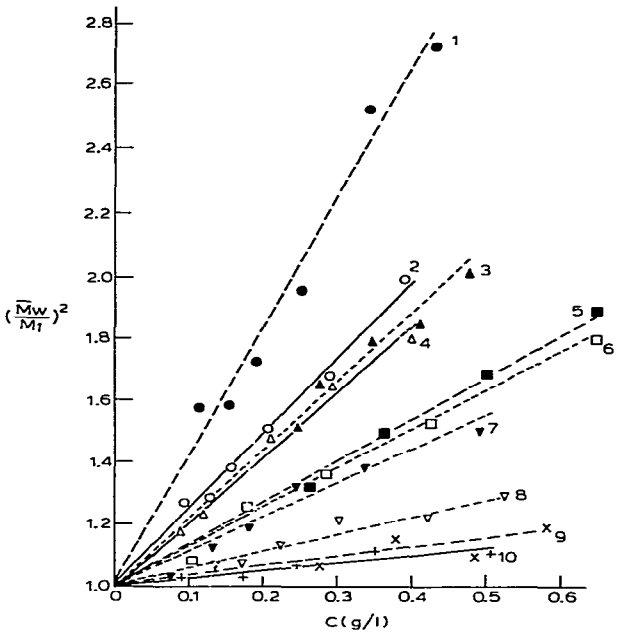

Fig. 5 The relative apparent molecular weight of KGDC at various concentrations and solvent conditions. See table 4 for a description of the solvent conditions and the symbols used in this figure.

4. Discussion

4.1. Physical properties of KGDC

The molecular weight we observed is in excellent agreement with that obtained in earlier sedimentation equilibrium studies [21] performed under conditions that suppressed the self-association.

Table 4

The effects of solvent components on the self-association of KGDC

Solvent	Composition	pH	T (°C)	K_1 [a] (l/g)	BM_1 [c] (l/g)	Concentration range examined (g/l)	Symbols used in fig. 5
0.1 M KCl		7.25	25	1.03±0.07	0.17	0.1–0.5	●
0.1 M KCl		6.8	25	0.33±0.01	0.085	0.25–0.7	■
0.1 M KCl		6.3	25	0.08±0.05	0.025	0.25–0.6	×
0.1 M KCl, 0.1 ionic strength phosphate		7.9	25	0.61±0.04	0.16	0.1–0.4	○
0.1 M KCl, 0.1 ionic strength phosphate		7.5	25	0.52+0.03	0.11	0.1–0.4	△
0.1 M KCl, 0.1 ionic strength phosphate		7.0	25	0.19±0.01 [b]	0.057	0.1–1.7	
0.1 M KCl, 0.1 ionic strength phosphate		6.5	25	0.07±0.01	0.022	0.1–0.5	+
0.1 M KCl, 0.1 ionic strength phosphate		7.0	20	0.14±0.02	0.058	0.1–0.5	▽
0.1 M KCl, 0.1 ionic strength phosphate		7.0	30	0.55±0.04	0.058	0.2–0.5	▲
0.3 M KCl, 0.1 ionic strength phosphate		6.9	25	0.27±0.04	0.025	0.1–0.5	▼
0.5 M KCl, 0.1 ionic strength phosphate		6.8	25	0.31±0.01	0.014	0.1–0.7	□

[a] These results were calculated from eq. 2.
[b] Table 3 has a more complete analysis of this datum.
[c] See text for the calculation of these values.

Nevertheless, the radius of gyration of KGDC determined in our measurements, 14 ± 1 nm, is larger than predicted by estimates of the molecular radius based on electron microscopy of the negatively stained KGDC (12.5 nm) [22] or the assumption of close packing of spherical components (11.5 nm) as described by Reed [1]. A radius of gyration of 14 nm is consistent with the sedimentation coefficient $s_{20,w} = 40$ S found by us and others [3]. If the commonly used solvation value of 0.2 g solvent species bound/g protein is accepted [14] then the Stokes radius (17.3 nm) of the 40 S particle corresponds to a sphere having a radius of gyration [14] of 13.4 nm. It thus appears that KGDC assumes a somewhat more expanded configuration in solution than is observed by electron microscopy.

4.2. Self-association of KGDC

Although the physiological impact of the self-association of KGDC is not clear, the self-association is substantial under physiological conditions (see table 1 and fig. 3), and may have an effect on the complex's enzymatic activity.

The choice of indefinite self-association over dimerization as the model describing the behavior of KGDC under the conditions of our experiments was suggested by the estimate of $Z_1 \approx -700$ as an upper limit at neutral pH in the absence of added salt and the probability that a substantial portion, at least, of this charge was retained in the presence of salt. The dimerization model is incompatible with such a charge or even with $Z_1 \approx -200$ to -400 calculated by means of the indefinite self-association model (table 3). The small magnitude of BM_1 relative to K_1, the limited precision with which BM_1 was measurable, and the unavailability of independent measurement of Z_1 in the presence of added salt, led us to adopt a single value of Z_1, calculated from combined measurements in 0.1 and 0.5 M KCl at neutral pH and 25°C. The equilibrium constants given in table 4 were devised with the aid of a calculated second virial coefficient [20,23] based on this value of $Z_1 = -336$ at pH 7 and an assumed linear variation of the charge of KGDC with pH. The assumption had a significant effect only upon the variation of the self-association with pH. Fortunately, in these cases the relative contribution of the correction for non-ideality was small (17–30%) compared to the association constant [24] and did not vary markedly with pH.

The nature of the forces driving the self-association of KGDC is indicated by the effect of temperature changes on the association constant. The van't Hoff $\Delta H°$ equals 24 ± 7 kcal/mol^{-1} monomer added at pH 7.0 and 0.2 ionic strength. Under these conditions $G°$ equals -7.8 kcal mol^{-1} and $\Delta S° = 1.1 \times 10^2$ cal/mol^{-1} K^{-1}. The signs of $\Delta H°$ and $\Delta S°$ are indicative of hydrophobic bonding. Ion-pair formation, a possible alternative explanation [25,26], should be weakened by increasing electrolyte concentration. As the opposite is observed, this mechanism is unlikely. The magnitudes of $\Delta G°$, $\Delta H°$ and $\Delta S°$ are not extraordinary compared to the association of tobacco mosiac virus protein [20] or chymotrypsin [15], nor are the signs unusual for associating systems [26]. The values of $\Delta H°$ and $\Delta S°$ may indicate modest rather than very extensive regions of interaction between the KGDC molecules.

In addition to nonspecific hydrophobic interactions, the effect of pH upon the KGDC self-association may indicate the involvement of specific groups in the association. Experiments performed under conditions that eliminate contributions due to buffer ion concentration changes indicate $(\partial \ln K/\partial \ln H^+)_{I,T} = \Delta\nu_{H^+} = -1.12 \pm 0.07$ over the pH range 6.2–7.4 (H^+, activity of protons; I, ionic strength). This value of $\Delta\nu_{H^+}$, which implies a release of one proton per protomer added to the aggregate [27], differs slightly from the result obtained in the presence of phosphate buffer components where $(\partial \ln K/\partial \ln H^+)_{I,T} = 0.87 \pm 0.07$ over the same range. The difference between these two results, 0.25 ± 0.10, suggests a minor 'preferential' interaction [28,29] of phosphate ions with KGDC.

The effect of KCl on the association of KGDC is more difficult to interpret: the effect may be due to preferential or electrostatic interactions. Our experiments measured [28] $\Delta\nu_{KCl}(\text{pref}) = (\partial \ln K/\partial \ln KCl)_{pH} = 0.27 \pm 0.02$. This value of $\Delta\nu_{KCl}(\text{pref})$ may reflect binding of KCl, release of water during the association, or both. An electrostatic interpretation of the effect of added KCl on

the association [14,30] requires the assumption of a geometry for the KGDC molecule. An approximation of the complex geometry of KGDC [1,22] by a hard sphere or a solvent-permeated sphere model [14] yields quite different estimates of the charge, −100 for the former and −600 for the latter. (Note that the charge estimate of −336 calculated from the second virial coefficient is bracketed by these two values.) From the data it is not possible to choose between preferential interaction or the electrostatic effect as the correct interpretation of the influence of KCl on the self-association [31]. Nevertheless, $\Delta\nu_{KCl}$(pref) is an operationally useful quantity in that it permits convenient computation and prediction of the effect of KCl on the association.

The self-association of KGDC molecules by the indefinite model suggests multiple sites of interaction on the surfaces of these molecules. These sites may be important in the cellular localization of KGDC, for example on hydrophobic structures. From a thermodynamic point of view, the self-association stabilizes the KGDC in solution, thus hindering the dissociation of the KGDC molecule into its component enzymes and so perhaps serving a useful function to the cell.

The complexity and subtlety of even this simplest of the equilibria that govern the formation and function of KGDC highlight the care that is required in the analysis of the properties of the many physiologically important large multi-enzyme complexes.

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