Regulation of Glucose-6-Phosphate Dehydrogenase Activity in Sea Urchin Eggs by Reversible Association with Cell Structural Elements

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Abstract. In unfertilized eggs of the sea urchin, *Strongylocentrotus purpuratus*, glucose-6-phosphate dehydrogenase (G6PDH) associates with the particulate elements remaining either after homogenization or extraction of eggs with non-ionic detergent in low ionic–strength media. At physiological ionic strength, the extent of G6PDH binding to these particulate elements is proportional to the total protein concentration in the extracts. In fertilized eggs this association is prevented by one or more low molecular weight solutes. The dissociation is reversible, and there are no permanent modifications of either G6PDH or its particulate binding site that affect binding. After fertilization, the time course of dissociation of G6PDH from particulate elements is too fast to be caused by a change in intracellular pH, but it could be triggered, but not maintained, by an increase in the intracellular calcium concentration. Binding of G6PDH to the particulate fraction lowers its catalytic activity at all substrate concentrations. Therefore, release of the enzyme into the cytoplasm may be an important part of the suite of events causing metabolic activation of the egg at fertilization.

SEA urchin eggs contain the substrates and catalysts needed for active metabolism, but are dormant before fertilization. At fertilization, changes in the intracellular levels of Ca^{++} and H^+ relieve the egg from its metabolic restraints (5, 30). Two of the cellular changes attending fertilization are reduction of the pyridine nucleotides (4, 29), and increased carbohydrate metabolism (12, 15) by way of the hexose monophosphate shunt (12). Both phenomena are related to the activity of glucose-6-phosphate dehydrogenase (G6PDH),[1] which catalyzes the reaction: glucose-6-phosphate + $NADP^+$ = 6-phosphogluconate + $NADPH$ + H^+.

At fertilization there is a major reorganization of cell structure in the zygotes, which possibly plays a role in the activation of metabolic pathways needed for development. Since accelerated carbohydrate catabolism likely involves stimulated G6PDH activity (12), it is intriguing that G6PDH activity resides in the particulate fraction of homogenates prepared from unfertilized sea urchin eggs, but that it shifts to a soluble fraction in zygotes (11). Similar results were obtained in studies of the eggs and zygotes of the surf clam *Spisula solidissima* (10). In both cases, particulate G6PDH is released in a soluble form in homogenates by high ionic strength, alkaline pH, or low concentrations of the substrates or products of the G6PDH reaction. It was suggested that a rise in intracellular concentrations of substrates after fertilization solubilizes the G6PDH, and thereby increases its catalytic activity (12).

In the present study we address the following questions.

(a) Is the observed solubilization of G6PDH upon fertilization of sea urchin eggs a physiological event rather than an artifact of homogenization? (b) What at fertilization releases the enzyme from the particulate elements? (c) What are the consequences of binding to the enzyme's activity? A preliminary report of these data has been published (27).

Materials and Methods

Experimental Animals

Handling of Gametes

Spawning was induced by injection of 0.5 M KCl into the coelomic cavity. Injected females were placed, oral side up, atop a beaker filled with filtered seawater, and the eggs shed directly into the collection beaker. Egg jelly coats were removed as described previously (23). Sperm was collected "dry." In vitro fertilization was carried out by adding one part sperm to two thousand parts egg suspension. Samples giving <90% fertilization were discarded.

Enzyme Measurements

G6PDH activity was assayed as described by Langdon (16). The rate of NADPH production was followed by monitoring as a function of time either the absorption of light at 340 nm or the intensity of fluorescence at 460 nm of samples irradiated at 350 nm. A cuvette stirring device (Spectrocell, Inc., Oreland, PA) was used to maintain a homogenous suspension in the fluorometric assays of the activity of the particulate-bound enzyme.

G6PDH binding to the particulate fraction was assessed as follows. Suspensions of gametes (1–2%) were homogenized (Dounce tight-fitting

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1. *Abbreviations used in this paper*: G6P, glucose-6-phosphate; G6PDH, G6P dehydrogenase.

homogenizer, 15 strokes) in 50 mM 2-(N-morpholino)ethane sulfonic acid (MES) (pH 6.5, 4°C), 6 mM EGTA, and 20 mM KCl (MEK buffer), and then centrifuged rapidly in an Eppendorf microfuge (2–5 min, 12,000 g). The supernatant (S1) was removed, and the pellet resuspended either in 20 mM Tris–Cl (pH 8.0, 4°C) containing 0.1 M KCl (KCl buffer), or in 10 mM Pipes (pH 6.8, 4°C) containing 0.33 M glycine, 0.3 M potassium gluconate, 2 mM MgSO₄, and 2 mM EGTA (KGE buffer). The volume of KCl buffer or KGE buffer added to the pellet equalled the volume of S1. Both KCl buffer and KGE buffer solubilize all G6PDH bound to the particulate fraction. The suspension was then centrifuged again, and the supernatant (S2) was retained. S1 and S2 were then assayed for G6PDH activity, and the percentage of the total G6PDH activity (S1 + S2) contained in S2 is taken to be the percent of bound G6PDH in the original homogenate. Identical results were obtained when 50 mM Imidazole was substituted for 50 mM MES in the MEK buffer. Unless specified otherwise, zygote homogenates were prepared at 4 min after insemination.

Isolation of particulate-bound G6PDH was accomplished by homogenizing unfertilized eggs in MEK buffer, centrifuging, and then washing the pellet twice in MEK by resuspension and centrifugation.

When detergent extraction was used in place of homogenization, the buffers contained 0.5% Triton X-100. The samples were incubated for 15 min at 16°C with occasional gentle mixing, centrifuged (2–5 min, 12,000 g), and analyzed for the amount of G6PDH associated with the detergent-insoluble residue by the procedure outlined above for homogenized samples.

In experiments where high concentrations of chicken ovalbumin were included in the extraction buffer (KGE), 200 mg/ml stock solutions of ovalbumin were prepared as follows. 400 mg of ovalbumin were placed in a graduated conical centrifuge tube, KGE buffer was added until the volume of the mixture was ~1.5 ml, and the tube was then sealed with Parafilm. Dissolution of the protein was accomplished by gentle repeated inversion and twirling of the tube. The pH of the solution was then readjusted (from 5.4 to 6.8) very carefully, since an overshoot in the addition of base causes rapid and irreversible formation of a gel. The final volume of solution was then adjusted to 2 ml with the addition of KGE buffer. (The pH electrode was then treated with a solution of 1–5 mg/ml trypsin to prevent clogging of the electrode with ovalbumin.)

Cytoplasmic Protein Concentration Measurements

Cytoplasmic protein concentration was estimated from the amount of protein remaining soluble after ultracentrifugation (100,000 g, 2 h) of egg homogenates prepared in KGE medium. These determinations were made at various egg cell concentrations (from 1% to completely packed cells) to correct for possible dissociation of insoluble protein complexes, due to dilution with the homogenization medium, that would result in artifactually high estimates of dissolved protein in the cytoplasm. Cytoplasmic protein concentrations were calculated assuming that the cytoplasm comprises 61% of the volume of a sea urchin egg (8), and that cell packing efficiency is 76%. Protein concentration was measured in samples as described by Esen (6).

Results

Comparison of Homogenization with Detergent Extraction

A shift in the sedimentation pattern of G6PDH upon fertilization was observed in samples homogenized in low ionic strength, low pH buffers (MEK buffer; Table I, and references 1 and 11). Addition of detergent (0.5% Triton X-100) to MEK buffer destroyed cellular integrity, but the addition of sucrose (0.9 M) to make the Triton–MEK extraction medium isotonic produced an insoluble residue retaining the spherical characteristics of the egg; the data in Table I indicate that the G6PDH binding sites remain with the low salt–insoluble residue or the detergent-insoluble residue from unfertilized eggs, but the enzyme is not associated with this insoluble component after fertilization. Intact unfertilized eggs suspended in MEK buffer containing 0.9 M sucrose did not bind exogenously added sea urchin G6PDH, which eliminates the possibility that G6PDH released upon extraction binds to sites on the external surface of the egg.

Table I. Comparison of Homogenization with Detergent Extraction of S. purpuratus Eggs

Sample	Treatment*	Buffer	Bound G6PDH
			%
Unfertilized	H	MEK	62
Unfertilized	DE	MEK/Sucrose	55
Fertilized	H	MEK	11
Fertilized	DE	MEK/Sucrose	6
Unfertilized	H	KGE	5
Unfertilized	DE	KGE	3

* H refers to homogenization of the samples. DE refers to 15-min detergent extraction of the samples at 16°C. All samples were centrifuged at 12,000 g for 2 min, and analyzed for the extent of G6PDH binding as described in Materials and Methods.

This binding was not seen in high ionic strength media. In KGE buffer, a medium that approximates some of the solution conditions of the egg's cytoplasm, the amount of G6PDH associated with the particulate fraction of unfertilized eggs was only 5% (Table I).

The homogenates prepared in KGE have an ionic composition similar to egg cytoplasm, but do not contain soluble protein levels similar to what may be present in vivo. Other workers have shown that high concentrations of protein can have dramatic effects on the thermodynamics and kinetics of cellular processes (7, 19, 20). Since the concentration of protein in the cytoplasm of unfertilized S. purpuratus eggs is 67.1 ± 11.6 mg/ml by our measurements, we assessed the effects of protein on G6PDH distribution by adding ovalbumin to the KGE extraction buffer. We used a high volume ratio of eggs to extraction medium (1:1) in these experiments to further enhance the concentration of protein in solution. Ovalbumin was selected as additive because it is a globular protein that does not self-associate at high concentrations, has no known enzymatic activity, and has not been demonstrated to remove significant quantities of solutes from solution as do serum albumins. It is, however, a glycoprotein, and therefore may not be completely representative of proteins in the aqueous cytoplasm.

The results (Fig. 1) indicate that G6PDH binding increases in proportion to the total concentration of protein in KGE detergent extracts of unfertilized eggs, but does so to a much lesser extent in extracts of fertilized eggs. When extrapolated to zero protein concentration, G6PDH binding in eggs is nearly identical to that in embryos. In a control experiment, 10 μM NADP, a condition known to elute G6PDH from particulate elements (1), reversed the binding of G6PDH in unfertilized eggs extracted at a protein concentration of 90 mg/ml, demonstrating that this detergent-extracted residue can release the enzyme at high protein concentrations.

Ultracentrifugation and Gel Filtration of Homogenates

The shift of G6PDH to the supernatant noted above is not because the enzyme binds to a smaller particle in embryos, since centrifugation of the zygote homogenate at 100,000 g for 1 h shows that 77% of the enzyme remains in solution (as compared to 75% soluble enzyme after 2 min at 12,000 g). Furthermore, as seen in Fig. 2, two peaks of G6PDH activity appear in the gel filtration elution profile of the chromatographed homogenates, an early peak at the column's void volume, and a later, broader peak, well included into the column matrix. The void volume peak, which
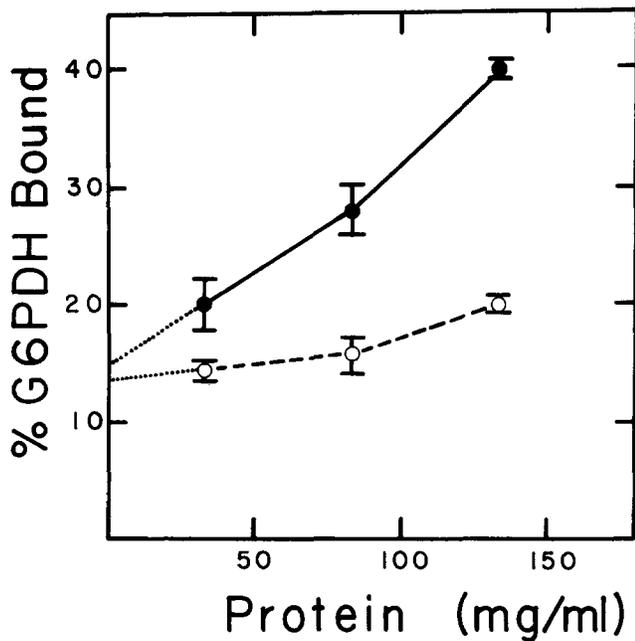
Figure 1. Protein concentration dependence of the association of G6PDH with egg cell structural elements. 50% suspensions of *S. purpuratus* eggs (closed symbols) or zygotes (open symbols) were extracted with KGE buffer containing 0.5% Triton X-100, and the concentration of protein was fortified by adding ovalbumin to yield the total protein concentrations indicated on the abscissa. After incubating 15 min at 16°C, the extracts were centrifuged, and the percent G6PDH bound to the particulate elements was determined as described in the text. Zygotes were briefly treated with 1 M urea solutions after insemination to remove the fertilization membrane, a barrier that has been shown to retain 95% of the protein in permeabilized sea urchin zygotes (26). Controls with homogenized samples showed that this treatment does not perturb the extent of binding of G6PDH in the zygote cells. The data shown are from two experiments assayed in duplicate; error bars indicate the range around the mean values.

most likely contains the particulate-associated enzyme, comprises 55% of the total activity in the chromatographed homogenate from unfertilized eggs (Fig. 2 *A*), whereas the corresponding peak from the zygote homogenate (Fig. 2 *B*) contains 31% of the total activity. This agrees well with the sedimentation analysis of G6PDH binding in these samples, which showed 57% of the enzyme bound in unfertilized eggs, and 24% bound in the zygotes. The chromatogram of the zygote supernatant (Fig. 2 *C*) contained no activity in the void volume, instead the zygote supernatant enzyme eluted only slightly ahead of bromophenol blue, indicating that the zygote enzyme is soluble as this latter term is generally understood.

Time Course of G6PDH Release after Fertilization

We monitored the extent of enzyme binding as a function of time after insemination. We found (Fig. 3, *inset*) that 50% of G6PDH solubilization occurs within 30 s after the addition of sperm, indicating that G6PDH release is one of the very early events involved with metabolic activation of eggs. Once dissociated, the enzyme remains soluble throughout the first cell cycle in the zygote (Fig. 3), in contrast with the results obtained with *Spisula* eggs (10).
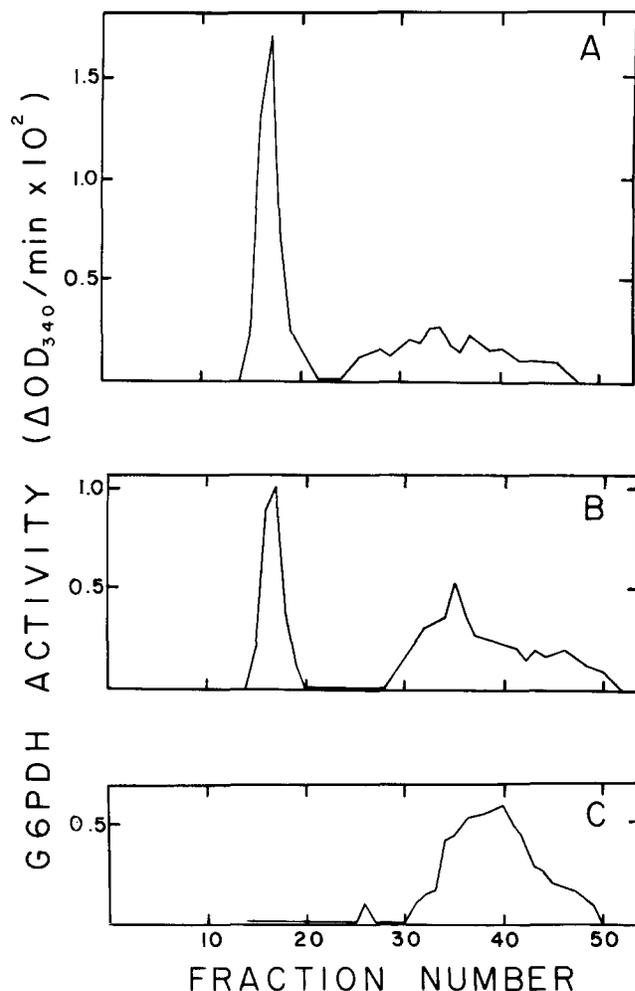

Figure 2. Analysis of G6PDH solubility in unfertilized and fertilized egg homogenates by agarose gel filtration chromatography. 1-ml samples of unfertilized egg homogenate (*A*), fertilized egg homogenate (*B*), or fertilized egg supernatant (*C*) were chromatographed on a 2.5 × 18 cm column of Bio-gel A-15M (Bio-Rad Laboratories, Richmond, CA). The concentration of eggs before homogenization was 1.5%. The column elution buffer contained 50 mM Imidazole-Cl, pH 6.5, 6 mM EGTA, and 20 mM KCl, and the temperature was maintained at 4°C. Each fraction contained 1.3 ml. After chromatography, the pH and the KCl concentration of each fraction was brought to 8.0 and 100 mM respectively in order to release any bound G6PDH activity. Fraction 17 represents the void volume and fraction 46 represents the included volume as determined by chromatography of blue dextran and bromophenol blue, respectively. The peak of baker's yeast G6PDH was at fraction 42.

Effects of Ca_i^{++} and pH_i Changes on G6PDH Release

The timing of the G6PDH change after fertilization (Fig. 3) suggests that G6PDH dissociation may be related to changes in Ca_i^{++}, rather than to changes in pH_i. In support of this we find that eggs activated with 25 μM Ca^{++}-ionophore A23187 in sodium-free seawater, a condition which increases Ca_i^{++} but prevents the pH_i rise (24), show a transformation of the enzyme from 65% bound to 10% bound by 2 min after addition of ionophore. Furthermore, preventing the pH_i rise in zygotes by transferring them to sodium-free seawater within 30 s of insemination does not inhibit solubilization (Fig. 3, *inset*). Finally, raising the intracellular pH of unfer-
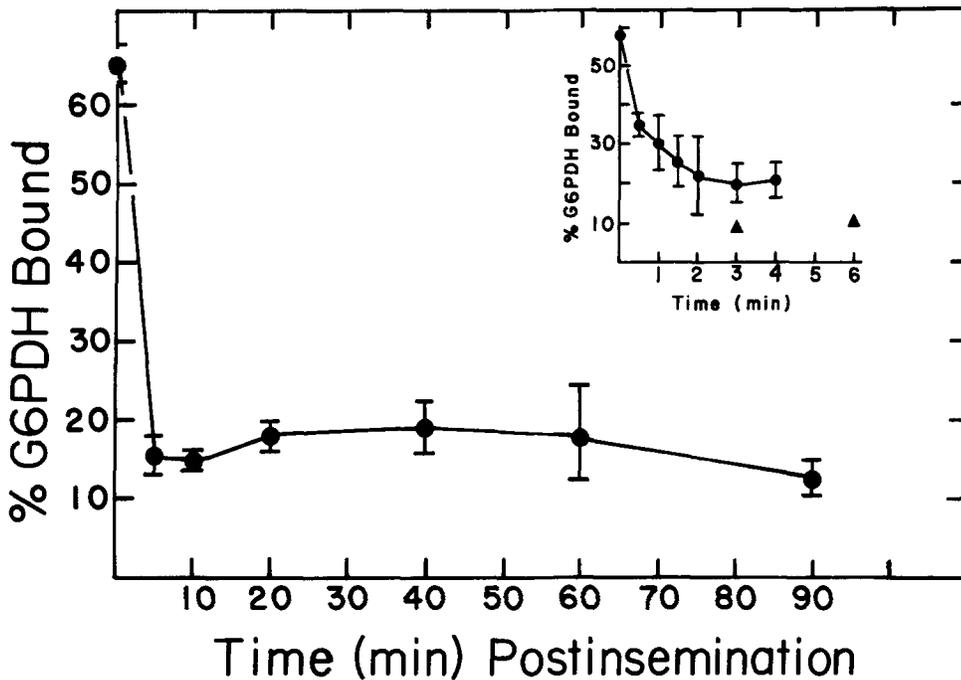
Figure 3. Time course of G6PDH release from the particulate elements after fertilization. A 1.5% suspension of *S. purpuratus* eggs was inseminated, and at the times after insemination indicated on the abscissa, samples were homogenized in MEK buffer and analyzed for G6PDH binding as described in the text. The circles represent eggs that were maintained in filtered seawater throughout the experiments, whereas the triangles represent samples that were transferred to sodium-free seawater within 30 s after fertilization. Three washes with sodium-free seawater insured complete removal of external sodium from the zygotes. The samples were maintained at 16°C; cells were beginning to divide by 90 min after insemination. Data presented are the mean of two experiments; error bars show the range of values obtained. The inset contains an expansion of the early time course of this phenomenon.

tilized eggs without affecting Ca_i^{++}, by incubating eggs in seawater containing 20 mM ammonium chloride, pH 8.0, for 10 min, or in pH 9.0 seawater for 10 min (13), fails to release bound G6PDH (data not shown).

Dialysis of Egg and Zygote Homogenates

Samples of fertilized eggs homogenized in MEK buffer, and then dialyzed for 4 h against 100 vol of MEK buffer at 4°C, with one change of buffer after 2 h, were assayed for G6PDH binding to determine if dialysis reverses the release of the enzyme from the particulate fraction. The results (Table II) show that G6PDH binding to the particulate fraction after dialysis had increased almost to the same level as found in unfertilized eggs, indicating that the G6PDH dissociation is freely reversible upon removal from the zygote homogenate of some dialyzable component. Dialysis of unfertilized egg extracts against MEK buffer did not influence G6PDH binding.

Table II. Release and Re-binding of G6PDH upon Dialysis of Egg and Zygote Homogenates

Sample	Dialysis buffer	%G6PDH bound	
		Not dialyzed	Dialyzed
Egg homogenate	MEK	78	81
Zygote homogenate	MEK	32	63
Egg homogenate	Zygote supernatant	80	24
Particulate G6PDH	Zygote supernatant	97	33
Egg homogenate	Egg supernatant	62	65

The details of the dialysis protocol are given in the Results section. All samples were dialyzed against a minimum of 100 vol of dialysis buffer for at least 4 h.

When a crude homogenate of unfertilized eggs was placed in a dialysis tube and dialyzed for 4 h against 100 vol of a supernatant from centrifuged zygote homogenates, G6PDH was released from the structural elements of the unfertilized egg (Table II, line *3*). The same result was obtained when a suspension of isolated, particulate-bound G6PDH was placed in a dialysis bag and dialyzed against the zygote supernatant (100 vol), demonstrating that the dialyzable solute(s) acts on the bound enzyme directly, rather than on a soluble component contained within the egg homogenate.

Ability of G6PDH and the Particulate Fraction from Unfertilized or Fertilized Eggs to Reassociate In Vitro

To test for permanent modification of either G6PDH or its particulate binding site, we separated soluble G6PDH from enzyme-free particulate fractions of *S. purpuratus* eggs both before and after fertilization by homogenizing samples in KCl buffer and centrifuging (2 min, 12,000 g). The G6PDH-containing supernatants were passed through Biogel P-10 columns equilibrated with MEK buffer to exchange the KCl medium for the MEK medium (9). The enzyme-free pellets were washed three times by centrifugation and resuspension with KCl buffer, and then washed twice in MEK buffer. Various combinations of these soluble and particulate fractions were incubated together in MEK buffer for 15 min at 16°C, and then assayed for reassociation.

We found that the unfertilized egg enzyme binds equally well to the particulate fraction isolated either from unfertilized eggs (78% bound) or from zygotes (80% bound); likewise the fertilized egg enzyme binds equally well to the particulate fraction isolated either from unfertilized (82%

1512

bound) or fertilized (74% bound) eggs. We conclude, therefore, that no permanent modification occurs in either G6PDH or the particulate fraction to effect dissociation of the enzyme after fertilization.

Cross-species Binding Studies

Soluble G6PDH and G6PDH-free particulate fractions were prepared from unfertilized eggs of *S. purpuratus* and *L. pictus* and equilibrated with MEK buffer, following the procedure outlined in the preceding experiment. Mixing these fractions in various combinations and assaying G6PDH binding shows a remarkable lack of specificity in rebinding of the enzymes to the structural elements of these two species (Table III), i.e., either sea urchin enzyme is equally capable of binding to the particulate fraction of either species. The enzyme isolated from baker's yeast also bound to either of the sea urchin egg particulate elements to a similar extent, even though G6PDH is assumed to be a soluble enzyme in yeast cells (17, 28). The information for G6PDH binding must therefore reside in the sea urchin egg cell architecture, rather than in the structure of the sea urchin enzymes per se.

Kinetics of Soluble and Particulate G6PDH

The G6PDH reaction velocities of soluble and particulate G6PDH were measured as a function of glucose-6-phosphate (G6P) and of NADP (Fig. 4). At any concentration of substrate or coenzyme the ability of G6PDH to carry out catalysis is reduced in the presence of the particulate fraction. The apparent Michaelis constants (Eadie-Hofstee analysis) for G6P and NADP are, respectively, 39.3 μM and 1.5 μM for the soluble enzyme, and 203 μM and 4.1 μM for the particulate enzyme. The Michaelis constants for the soluble enzyme are similar to those reported for G6PDH in *Spisula* eggs (10).

Discussion

A number of enzymes once characterized as soluble have been found to bind to structural elements of cells, with modification of their activity as a consequence (reviewed in references 19 and 31). Wilson coined the term "ambiquitous" to

Table III. Cross-species Binding Studies

Species		
Enzyme	Particulate fraction	G6PDH Bound
		%
S. purpuratus	*S. purpuratus*	63
S. purpuratus	*L. pictus*	60
L. pictus	*L. pictus*	55
L. pictus	*S. purpuratus*	64
Yeast	*S. purpuratus*	63
Yeast	*L. pictus*	54

Preparation of the enzyme and the particulate fractions was as follows. Eggs were homogenized in KCl buffer, and the soluble G6PDH was separated from the enzyme-free particulate fraction by centrifugation (2 min, 12,000 g). The supernatant was removed, and equilibrated with MEK buffer by the centrifuge desalting technique of Helmerhorst and Stokes (9); yeast G6PDH was likewise equilibrated with MEK buffer. Particulate fractions were washed three times with KCl buffer to insure removal of all bound G6PDH, and then twice with MEK buffer by centrifugation and resuspension. The combinations of enzyme and particulate fractions listed above were incubated for 15 min at 16°C, then assayed for extent of rebinding as described in Materials and Methods.

describe such enzymes, and he postulated that these interactions might play an important role in metabolic regulation (31). The previously observed solubility of these enzymes may have resulted from extraction conditions not completely representative of the intracellular milieu.

Ionic strength of the medium, in particular, profoundly affects association of enzymes with structural elements, most often with the result that physiological ionic strengths renders these enzyme soluble (10, 11, 14, 21, 25). This was observed for the binding of G6PDH to egg structural elements, but restoring the concentration of protein in the extraction medium to that characteristic of the cytoplasm (67.1 mg/ml by our measurements, but cf. Clegg [3] for alternative interpretations of protein "solubility" in situ) offsets the salt-induced release of the enzyme. Other protein assembly processes respond similarly to total protein concentration (18), and decreases in the ideality of the solvent resulting from the increased excluded volume of the system at high protein concentration have been postulated to underly these phenomena (18, 20).
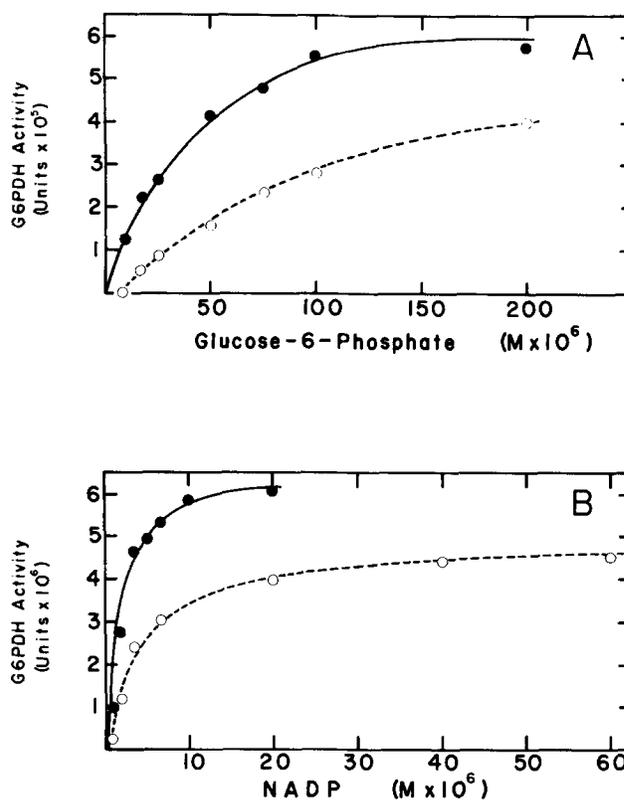

Figure 4. Kinetics of G6PDH in the presence of or in the absence of sea urchin egg particulate elements. Equal volumes of a suspension of *S. purpuratus* egg particulate fraction containing bound G6PDH were centrifuged, and the pellets were then resuspended in either MEK buffer, or in KCl buffer to obtain the particulate enzyme (open circles) or the soluble enzyme (filled circles), respectively, of equal total G6PDH concentrations. The latter suspension was centrifuged to remove all particulate elements before use in the assays. These preparations were then assayed for G6PDH activity as a function of G6P concentration at a fixed NADP concentration of 0.04 mM (*A*), or as a function of NADP concentration at a fixed G6P concentration of 0.417 mM (*B*). The assay buffer contained 50 mM Imidazole-Cl, pH 7.0, and 7 mM MgCl$_2$. The assay temperature was 16°C, and 1 U of activity corresponds to the reduction of 1 μmol of NADP per min.

A high concentration of protein does not artifactually force G6PDH binding since the enzyme in the zygote extracts does not associate with the structural elements of the fertilized egg, even at high protein concentrations (Fig. 1). Furthermore, the enzyme bound to structural elements of the unfertilized egg solubilizes at low concentrations of cofactor NADP in the presence of high protein concentrations. We therefore conclude that the low ionic–strength media used in most of our studies, while unphysiological, preserves many weak interactions existing in cells, including G6PDH binding to structure.

Other approaches to verify or refute these biochemical results, such as antibody localization at the ultrastructural level, need to be done. However, similar problems of preserving weak interactions between proteins during the fixation procedures could confound such investigations.

Intracellular Factors Causing G6PDH Release

The observation that alkaline pH reverses G6PDH binding to structural elements in vitro (1) is not relevant in vivo since G6PDH dissociation precedes intracellular alkalinization (24), and since blocking cytoplasmic alkalinization by removing extracellular sodium does not affect release. Both the kinetics of dissociation after fertilization, and the induction of release by the calcium ionophore A23187 suggest that the shift in G6PDH locale arises from the transient Ca_i^{++} elevation after fertilization (22), but the persistent solubility of the enzyme in zygotes when the intracellular calcium concentration falls almost to the unfertilized egg level (22) indicates that calcium acts indirectly. Our data suggest that Ca^{++} might cause production of a low molecular weight compound that acts on the bound enzyme complex directly, effecting dissociation. Preliminary investigations into this factor's identity indicate that it is not NADP, NADPH, or G6P, since the capacity of zygote supernatant to dissociate the enzyme in unfertilized egg homogenates is retained if the supernatant is treated with alkaline phosphatase, under conditions where G6P and NADP are hydrolyzed, or with 0.1 mM phenazine methosulfate, which oxidizes all NADPH present.

Barber et al. found changes in the electrophoretic mobility of G6PDH isozymes in *S. purpuratus* at fertilization, and proposed a posttranslational modification to the enzyme (2). This change apparently does not influence binding of the enzyme to cell structure, since our data show that binding between embryonic G6PDH and particulate fraction occurs when low ionic–strength media are free of cellular metabolites.

Role of G6PDH Binding Changes

What advantages might binding of G6PDH to cell structure confer upon egg cells? One possibility is that binding localizes the enzyme to areas of the cell in greatest need of the products of its activity, e.g., NADPH. However, data from immunofluorescent studies (not shown here) using a polyclonal antibody against sea urchin egg G6PDH show no discrete cellular localization of G6PDH in unfertilized *S. purpuratus* eggs, arguing against this hypothesis. Alternatively, binding may entail changes in the enzyme's conformation that modify its catalytic activity, and in support of this we found that the presence of the particulate fraction in the assay

mixture slowed the rate of the reaction. Gel filtration and ultracentrifugal studies provide strong evidence that the majority of the enzyme in eggs is bound and that in zygote cells it is free in solution, not attached to a smaller particle of reduced sedimentibility. Therefore, G6PDH activity after fertilization is unhindered by association with structural elements. If, as has been proposed (12, 15), the majority of carbohydrate catabolism in early zygotes occurs by way of the hexose monophosphate shunt, then the untrussing of G6PDH at fertilization may be one of the primary events that terminate the egg cell's dormancy.

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