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The molecular weight of rat brain hexokinases

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SUMMARY

The soluble hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) has been found to aggregate under conditions of low ionic strength. It is suggested that such aggregation offers an explanation for the heterogeneity in sedimentation velocity observed by Teichgräber and Biesold [*J. Neurochem.*, 19, 895 (1972)], which is in contrast to the homogeneous sedimentation properties observed in our laboratory. When aggregation is precluded, the soluble hexokinase sediments with a velocity compatible with a molecular weight of approximately 98 000, which is also the molecular weight of the purified particulate hexokinase. Other evidence supporting the essential similarity of the soluble and particulate enzymes is reviewed.

Approximately 80% of the total hexokinase (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1) activity in rat brain homogenates is particulate[1,2] being bound to the outer mitochondrial membrane[3]. Several reports are consistent with the view that the soluble and particulate enzymes represent identical protein molecules; *e.g.* there was no significant difference in electrophoretic behavior[4], or in chromatographic and kinetic properties[5,6]. Furthermore, the molecular weight of 96 000–99 000, reported for the soluble enzyme[7], is in excellent agreement with that found for the purified particulate enzyme[8]. Recently, however, it has been reported that the soluble enzyme exhibited heterogeneity in molecular weight as determined by sucrose density gradient centrifugation[9]. This was in contrast with results consistently obtained in our laboratory which indicated the soluble enzyme to be homogeneous in sedimentation behavior on sucrose density gradients. We have further investigated the situation and wish to describe some aggregation properties of the soluble hexokinase which may lead to a resolution of these conflicting reports.

The soluble enzyme was prepared as previously described[10] except that 0.05 M sucrose was used as the homogenizing medium to permit layering of the sample on 5–20% sucrose gradients prepared according to Teichgräber and Biesold[9]. Samples were concentrated approximately 3-fold in dialysis bags covered with Aquacide (Calbiochem). Pig heart lipoyl dehydrogenase (NADH:lipoamide oxidoreductase, EC 1.6.4.3) (Sigma Chemical Co.), which has a molecular weight of approximately 110 000[11], was used as a reference protein on the sucrose gradients, and was measured by the fluorescence of its flavin prosthetic group (activation, 455 nm, emission, 530 nm). Hexokinase activity was determined spectrophotometrically as previously described[8].

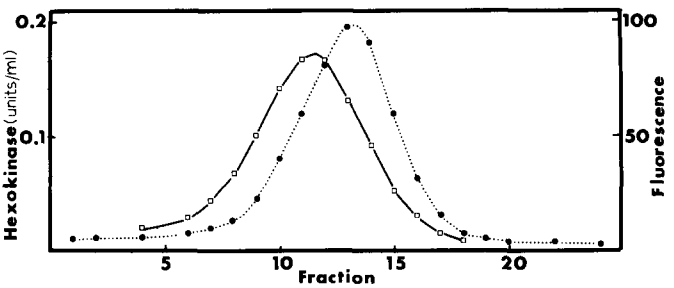

Fig. 1. Sucrose density gradient centrifugation of soluble rat brain hexokinase. Centrifugation was at 40 000 rev./min in a Beckman SW50.1 rotor for 16 h. Fractions were collected by puncturing the bottom of the tube, Fraction 1 being the bottom of the gradient. Hexokinase activity (●· · · ·●) and the marker protein, lipoyl dehydrogenase (□ —— □), were determined on the fractions.

As shown in Fig. 1, sucrose density centrifugation of soluble hexokinase resulted in a single peak of activity, with a sedimentation velocity compatible with a molecular weight of approximately 98 000, in agreement with previous reports[7,8]. The higher value (114 000) reported by Teichgräber and Biesold[9] may be due to the fact that they used a much higher molecular weight reference protein (catalase, molecular weight 250 000) in the gradient. It is clear from the results in Fig. 1 that the sedimentation velocity (and, by this criterion, the molecular weight) of the hexokinase is less than that of the pig heart lipoyl dehydrogenase.

In contrast to the report of Teichgräber and Biesold[9], no evidence of aggregation or inhomogeneity was ever seen. These authors observed a species with a molecular weight of 182 400 as well as smaller amounts of still larger aggregates, including some so large as to actually be pelleted under the centrifugation conditions used. Clearly any such higher molecular weight species should result in discernible hexokinase activity sedimenting ahead of the lipoyl dehydrogenase, but none was seen (Fig. 1).

The soluble enzyme was found to aggregate under conditions of low ionic strength. Dialysis against sucrose solutions of decreasing ionic strength resulted in increasing aggregation as measured by turbidity (absorbance at 600 nm) or relative amount of activity sedimented by centrifugation at 10 300 × *g* for 10 min. The results of an experiment using

potassium phosphate, pH 7.0, as the agent for increasing the ionic strength are presented in Fig. 2. Organic phosphates (*e.g.* glucose-6-*P*) or other inorganic salts (*e.g.* NaCl or KCl) were also effective at comparable ionic strengths. Results virtually identical with those shown in Fig. 2 were obtained when the particulate enzyme, solubilized by glucose-6-*P* treatment[2,4], was used instead of the soluble enzyme; this further indicates a similarity between the soluble and particulate forms.

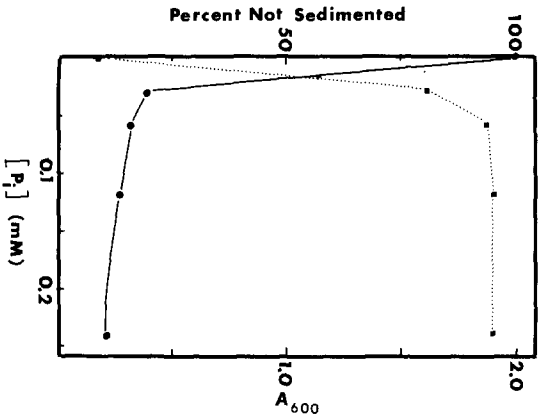

Fig. 2. Aggregation of soluble rat brain hexokinase at low ionic strengths. After 48 h dialysis of the soluble enzyme against 0.25 M sucrose containing the indicated concentration of potassium phosphate (pH 7.0), the turbidity of the sample was measured by the absorbance at 600 nm (●——●). Subsequently, the percentage of the total activity not sedimented by centrifugation at 10 300 ×*g* for 10 min was determined (■ · · · · ■).

We have found no evidence for heterogeneity in sedimentation behavior of soluble rat brain hexokinase when the enzyme has not been exposed to conditions conducive to aggregation. A closer examination of the procedures and results of Teichgräber and Biesold[9] suggests an explanation for their observation of such inhomogeneities. First of all, these authors indicate that they obtained soluble enzyme by centrifugation of a brain homogenate, prepared in 0.28 M sucrose, at 300 000 × *g* for 10 min. It is immediately obvious that soluble enzyme prepared in this manner could *not* be layered directly on a 5–20% sucrose gradient (5% sucrose ≈ 0.15 M) and it is therefore evident that these authors must have decreased the sucrose concentration to an acceptable level by some procedure not indicated in their paper. If in the process of reducing the sucrose concentration, the ionic strength is concomitantly reduced, the results of Fig. 2 clearly indicate that aggregation may be expected to occur. We suggest that this is the explanation for the heterogeneity in sedimentation behavior reported by Teichgräber and Biesold[9].

Furthermore, it appears that the ionic strength due to intracellular contents released by homogenization in 10 ml sucrose per g brain (our condition) is sufficient to prevent aggregation. However, Biesold and Teichgräber homogenized "two forebrains"

(we estimate the weight to have been 1–3 g) in 50 ml sucrose. Thus it is apparent that the ionic strength of their initial homogenate must also have been considerably lower than under our conditions. This could also contribute to the observed differences in aggregation state of the enzyme.

In summary, there is considerable evidence to support the view that the soluble and particulate hexokinases from rat brain are essentially identical with regard to several properties including molecular weight[4–8]. The contention that the soluble enzyme is heterogeneous in sedimentation properties[9] is not supported by experiments in our laboratory. We believe the aggregation of the enzyme at low ionic strengths could provide a reasonable explanation for the observed heterogeneity[9], and conclude that there is no convincing evidence for molecular weight differences between the soluble and particulate enzymes.

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