Short Communications

Activation and Solubilization of Particle-Bound Hexokinase in Rat Brain

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(Received 6 February 1967)

In recent years, remarkable progress has been made towards our understanding of the properties of hexokinases, their subcellular location in different organs and their metabolic function (see review by Walker, 1966). Little is known, however, about the particle-bound hexokinase that has been found in retina (Lowry, Roberts, Schulz, Clow & Clark, 1961), brain (Crane & Sols, 1953), muscle (Hernandez & Crane, 1966) and ascites-tumour cells (McComb & Yushok, 1959). Crane & Sols (1953) showed that, with particulate fractions from brain, treatment with lipase and deoxycholate results in a 50-fold increase in specific hexokinase activity, which is not accompanied by solubilization of the enzyme. Little solubilization of bound hexokinase could be found after extraction of brain tissue with organic solvents (Crane & Sols, 1953) or after freezing and thawing (Johnson, 1960). Balazs & Lagnado (1959) showed that several washings with 0.25M-sucrose did not remove significant amounts of hexokinase from rat-brain mitochondrial preparations. Hernandez & Crane (1966) solubilized the particle-bound hexokinase activity from pig heart by salt extraction between pH 4.6 and 7.0. Rose & Warms (1965) and Hernandez & Crane (1966), working with ascites-tumour cells and heart muscle respectively, solubilized the hexokinase by extraction with glucose 6-phosphate. In both cases it appears that the enzyme can recombine with subcellular particles.

The present investigation was made to find the conditions under which the brain particle-bound hexokinase can be completely solubilized. We found that part of the bound hexokinase activity was latent, so that it was also necessary to study the conditions for activation of this fraction. The hexokinase that is estimated under normal conditions can be solubilized with NaCl. Incubation with salt and Triton X-100 solubilizes all the activity, including the latent form.

Johnson (1960) reported that 75% of rat-brain hexokinase activity is located in the mitochondrial fraction. In our experiments, only 52% of the activity of the homogenate was found in this fraction (Biesold & Canzler, 1965). This discrepancy

can be explained by differences in the tissue fractionation procedure, which resulted in the latter case in a greater mitochondrial contamination of the nuclear fraction. This procedure has been published elsewhere (Balazs, Biesold & Magyar, 1963).

In the present study 67% of the hexokinase activity originally present in the rat-brain mitochondrial fraction was released into the supernatant after treatment of the material at 0° for 20 min. with 0.1M-NaCl at pH 5. At this pH the activity is completely released with 0.4M-NaCl. When the pH is increased, higher salt concentrations are necessary for complete release of the activity so that, at pH 7, 0.9M-NaCl gives complete solubilization. An increase in NaCl concentration from 0.1 to 0.9M at pH 7 raises the specific activity of the supernatant from 11 to 73 μ moles of glucose utilized/mg. of protein/hr., as compared with 6.7 μ moles of glucose/mg. of protein/hr. for the original mitochondrial preparation. Similar results were obtained when NaCl was replaced with KCl, MgCl₂ or (NH₄)₂SO₄. Our results show that brain hexokinase can be solubilized under the same conditions as the particle-bound heart hexokinase described by Hernandez & Crane (1966).

Incubation of brain mitochondrial preparations at 0° for 20 min. with 0.5% (w/v) Triton X-100 increases the activity from 232 to 457 μ moles of glucose utilized/g. wet wt./hr. It was concluded that at least 50% of the bound hexokinase activity is latent. Triton was much more effective than several other detergents tested under identical conditions. Cationic detergents tended to inhibit the enzyme. The activation of hexokinase by Triton was only observed with the particle-bound enzyme. The activity released into solution by salt extraction was not affected by subsequent addition of Triton.

Triton alone solubilized about 45–50% of the original hexokinase activity, but incubation with 0.9M-NaCl and 0.5% (w/v) Triton at pH 7 solubilized the original as well as the latent hexokinase activity. This result agrees well with the finding of Burkhard & Kropf (1964), who reported that

Table 1. *Effect of sodium chloride and Triton X-100 on the activation and solubilization of particle-bound hexokinase in rat brain*

The mitochondrial fraction prepared by the method of Balazs *et al.* (1963) was resuspended in 0.25 M-sucrose (1 ml. of suspension contained the equivalent of 300–400 mg. of original tissue). This suspension and all the incubation media contained 2-mercaptoethanol (10 mM). For estimation of activation and solubilization, 1 vol. of mitochondrial suspension was incubated with 9 vol. of the appropriate medium for 20 min. at 0°. The suspension was then centrifuged at 100000g for 1 hr. and hexokinase activity was determined in a sample of the supernatant. Hexokinase was assayed enzymically by using glucose 6-phosphate dehydrogenase by the method of Bennett, Drori, Krech, Rosenzweig & Abraham (1962). The composition of the test solution was as follows: glucose (25 mM), ATP (9 mM), Mg²⁺ (20 mM), NADP⁺ (1 mM), glycylglycine buffer, pH 8.0 (150 mM), and glucose 6-phosphate dehydrogenase in 100-fold excess, according to the activity of hexokinase. The NADP⁺ reduction was measured at 25° and 366 m μ . Protein was determined by the procedure of Rieder (1959).

Addition to incubation mixture	Activity		Solubilized activity	
	(μ moles of glucose/g. wet wt./hr.)	(% of original activity)	(μ moles of glucose/g. wet wt./hr.)	(% of original activity)
None	232	100	8	3
NaCl (0.9 M)	264	113	224	96
Triton X-100 (0.5%, w/v)	475	205	105	45
NaCl (0.9 M) + Triton X-100 (0.5%, w/v)	511	220	486	209

addition of salts increased the liberation of proteins from ox heart-muscle mitochondria with detergents. The results of a typical experiment of activation and solubilization by NaCl and Triton X-100 respectively are given in Table 1.

Triton X-100 solubilizes a large proportion of the proteins of the mitochondrial fraction (Crane & Sols, 1953; Brunngraber & Aquilar, 1962; Abood & Alexander, 1957), but, under these conditions, only part of the hexokinase is solubilized. From the different effects of salt and Triton on the liberation of hexokinase, specific binding sites may be involved. Hexokinase is not unspecifically adsorbed on the particulate fraction, since it cannot be released into the supernatant by several washings with 0.25 M-sucrose, or with 50 mM-tris-HCl buffer, pH 7, or by freezing and thawing in 0.25 M-sucrose.

In recent experiments, where a study was made of the distribution of hexokinase among subfractions of the brain mitochondrial preparation obtained by density-gradient centrifugation, the enzyme could not be precisely located. Activity was mainly found in subfractions that, according to electron-microscopic and enzymic evidence, contained, besides mitochondria, 'synaptosomes' and fragments of endoplasmic reticulum (D. Biesold, unpublished work). These results, together with those given above, still form an inadequate basis for the discussion of the roles of different modes of

occurrence of hexokinase in the regulation of glucose metabolism. We have no evidence whether the activity solubilized by salt and that activated by Triton represent the same enzyme. Preliminary kinetic studies have not revealed any significant difference in the K_m values for glucose.

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