THE LATENT HEXOKINASE ACTIVITY OF RAT BRAIN MITOCHONDRIA*

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PREVIOUS STUDIES (LOWRY ET AL., 1964; SACKTOR ET AL., 1966) HAVE INDICATED AN IMPORTANT ROLE FOR HEXOKINASE IN CONTROLLING THE CEREBRAL GLYCOLYTIC RATE. A MAJOR PORTION OF THE HEXOKINASE IN BRAIN IS FOUND IN THE MITOCHONDRIAL FRACTION (CRANE AND SOLS, 1953; JOHNSON, 1960). HEXOKINASE HAS ALSO BEEN FOUND IN THE MITOCHONDRIAL FRACTIONS FROM OTHER ANIMAL (E.G., HERNANDEZ AND CRANE, 1966; ROSE AND WARMS, 1967) AND PLANT (SALTMAN, 1953) TISSUES. THE PHYSIOLOGICAL SIGNIFICANCE OF A MITOCHONDRIAL LOCATION FOR HEXOKINASE, AND ITS POSSIBLE FUNCTION IN GLYCOLYTIC CONTROL, IS OF GREAT INTEREST.

THE RESULTS DESCRIBED IN THIS REPORT SHOW THAT LESS THAN HALF OF THE TOTAL HEXOKINASE IS READILY ASSAYABLE IN RAT BRAIN MITOCHONDRIA ISOLATED BY CONVENTIONAL TECHNIQUES, INDICATING THAT APPRECIABLE AMOUNTS OF ACTIVITY ARE LATENT, I.E., BOUND SO AS TO RENDER THEM INACCESSIBLE TO EXOGENOUS SUBSTRATE. TREATMENT WITH DETERGENTS, OSMOTIC SHOCK, OR FREEZE-THAW TREATMENTS CONVERT THE LATENT ENZYME TO AN ASSAYABLE STATE. THE LATENT HEXOKINASE IS ELECTROPHORETICALLY INDISTINGUISHABLE FROM THE "ASSAYABLE" ENZYME, AND BOTH ARE SUSCEPTIBLE TO ELUTION BY RELATIVELY LOW CONCENTRATIONS OF ATP AND GLUCOSE-6-PHOSPHATE (G-6-P).

HEXOKINASE WAS ASSAYED AT ROOM TEMPERATURE ACCORDING TO HERNANDEZ AND CRANE (1966). ONE UNIT IS DEFINED AS THAT AMOUNT OF ENZYME CATALYZING

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*IN CONDUCTING THE RESEARCH DESCRIBED IN THIS REPORT, THE INVESTIGATORS ADHERED TO THE "PRINCIPLES OF LABORATORY ANIMAL CARE" AS ESTABLISHED BY THE NATIONAL SOCIETY FOR MEDICAL RESEARCH.

THE FORMATION OF ONE μMOLE G-6-P PER MINUTE.

WHOLE BRAINS WERE REMOVED FROM ADULT MALE WISTAR-DERIVED RATS, 300-450 G, AFTER DECAPITATION UNDER LIGHT ETHER ANESTHESIA. BRAINS WERE KEPT ON ICE FOR THE SHORT PERIOD PRECEDING PREPARATION OF THE HOMOGENATE AS DESCRIBED IN TABLE I. THE FRACTION USED AS SOURCE OF THE MITOCHONDRIAL ENZYME CONTAINS PARTICLES OTHER THAN MITOCHONDRIA, BUT CENTRIFUGAL FRACTIONATION AND DENSITY GRADIENT EXPERIMENTS (TO BE DESCRIBED ELSEWHERE) INDICATED THAT NO APPRECIABLE AMOUNTS OF ACTIVITY WERE LOCATED IN PARTICLES OTHER THAN MITOCHONDRIA.

AS SHOWN IN TABLE I, TREATMENT OF THE PARTICULATE ENZYME WITH TRITON X-100 RESULTED IN AN INCREASE OF MORE THAN 100% IN THE AMOUNT OF ASSAYABLE ACTIVITY. TREATMENT WITH 1% SODIUM DEOXYCHOLATE GAVE SIMILAR RESULTS. INCREASES IN BOTH "SOLUBLE" AND "PARTICULATE" ACTIVITY WERE OBSERVED, INDICATING THAT THE DETERGENTS NOT ONLY CAUSED EXPOSURE OF MUCH OF THE LATENT ACTIVITY, BUT WERE PARTIALLY EFFECTIVE AT BRINGING ABOUT ITS SOLUBILIZATION. TREATMENT WITH G-6-P OR ATP RESULTED IN APPRECIABLE SOLUBILIZATION, BUT THESE AGENTS WERE LESS EFFECTIVE THAN DETERGENTS AT CAUSING EXPOSURE OF THE LATENT ACTIVITY. THE COMBINATION OF TRITON PLUS ATP OR G-6-P WAS EXTREMELY EFFECTIVE AT EXPOSING AND SOLUBILIZING HEXOKINASE, WITH THE OBSERVED TOTAL ACTIVITY BEING ABOUT 2 1/2 TIMES THAT FOUND FOR THE UNTREATED PREPARATION. TREATMENT OF SOLUBLE ENZYME WITH DETERGENT, ATP, OR G-6-P DID NOT RESULT IN INCREASED ACTIVITY INDICATING THAT THE OBSERVED INCREASE IN ACTIVITY SHOWN IN TABLE I WAS NOT DUE TO ANY "ACTIVATION" OF THE ENZYME BY THESE AGENTS.

RAPID FREEZE (LIQUID N_2)-THAW TREATMENTS OR OSMOTIC SHOCK BY RESUSPENSION OF THE PARTICULATE ENZYME IN DISTILLED WATER CAUSED LARGE (85-170%) INCREASE IN ASSAYABLE PARTICULATE ACTIVITY WITH ONLY SLIGHT INCREASE IN THE SOLUBLE ACTIVITY. THUS FREEZE-THAW AND OSMOTIC SHOCK ARE EFFECTIVE AT EXPOSING THE LATENT ACTIVITY BUT DO NOT PROMOTE APPRECIABLE SOLUBILIZATION.

TABLE I

EFFECT OF TRITON X-100, G-6-P, AND ATP ON PARTICULATE RAT BRAIN HEXOKINASE[A]

ADDITION	UNITS SOLUBLE	UNITS PARTICULATE	UNITS TOTAL
WATER	0.101	0.304	0.405
0.5% TRITON X-100	0.377	0.453	0.830
1.2 MM G-6-P	0.405	0.124	0.529
8 MM ATP	0.400	0.107	0.507
0.5% TRITON X-100, 1.2 MM G-6-P	0.905	0.112	1.017
0.5% TRITON X-100, 8 MM ATP	1.029	0.073	1.102

A. THE BRAINS FROM TWO RATS (2.98G) WERE HOMOGENIZED IN 10 VOLUMES (30 ML) 0.25 M SUCROSE AND THE HOMOGENATE CENTRIFUGED AT 1000 X G X 15 MIN. THE SUPERNATANT WAS THEN CENTRIFUGED AT 41300 X G X 15 MIN. AFTER DECANTING THE SUPERNATANT, THE PELLET WAS RESUSPENDED IN 6 VOLUMES (18 ML) 0.25 M SUCROSE. ALIQUOTS (0.8 ML) OF THE RESUSPENDED PELLET WERE ADDED TO TUBES CONTAINING 0.2 ML OF WATER OR STOCK SOLUTIONS OF ADDITIONS TO GIVE THE FINAL CONCENTRATIONS INDICATED. TOTAL VOLUME, 1.0 ML. AFTER MIXING, THE TUBES WERE INCUBATED 30 MIN. AT 28°, THEN CENTRIFUGED AT 41300 X G X 30 MIN. "SOLUBLE" AND "PARTICULATE" ACTIVITY REFERS TO THE SUPERNATANT AND PELLET, RESPECTIVELY. PELLETS WERE THOROUGHLY RESUSPENDED IN 0.25 M SUCROSE BEFORE ASSAY.

ISOZYMES OF HEXOKINASE HAVE BEEN SHOWN TO EXIST IN VARIOUS RAT TISSUES (KATZEN AND SCHIMKE, 1965). ON THE BASIS OF THE RESULTS SHOWN IN TABLE I AND OTHER EXPERIMENTS, NOT REPORTED HERE, THE CONDITIONS USED BY KATZEN AND SCHIMKE WOULD NOT BE EXPECTED TO SOLUBILIZE APPRECIABLE QUANTITIES OF THE LATENT HEXOKINASE. THEREFORE, TO TEST THE POSSIBILITY THAT THE LATENT HEXOKINASE MIGHT BE A DIFFERENT ISOZYME FROM THE MORE READILY EXTRACTED ENZYME STUDIED BY THESE AUTHORS, EXTRACTS OF SOLUBLE "ASSAYABLE" ENZYME WERE PREPARED BY HOMOGENIZATION IN 0.25 M SUCROSE, WHILE APPRECIABLE AMOUNTS OF LATENT ENZYME WERE THEN SOLUBILIZED BY REHOMOGENIZING IN 0.20 M SUCROSE-1% TRITON-20MM ATP, PH 7.0. HORIZONTAL STARCH GEL ELECTROPHORESIS WAS PERFORMED ESSENTIALLY ACCORDING TO KATZEN AND SCHIMKE (1965). THE HEXOKINASE ACTIVITY IN THE BRAIN EXTRACTS MIGRATED AS A SINGLE ISOZYME BAND (TYPE I IN THE NOMENCLATURE OF KATZEN AND SCHIMKE) WHILE CONTROL EXTRACTS FROM OTHER TISSUES (E.G., LIVER) SHOWED THE EXPECTED ISOZYME

PATTERNS (KATZEN AND SCHIMKE, 1965). THIS INDICATES THAT THE LATENT HEXOKINASE IS, LIKE THE "ASSAYABLE" HEXOKINASE, THE TYPE I ISOZYME.

MANSOUR ET AL., (1966) REPORTED THAT HEART MUSCLE PHOSPHOFRUCTOKINASE UNDERGOES A CONVERSION FROM A SOLUBLE, ACTIVE FORM TO A PARTICULATE, INACTIVE FORM WHEN HOMOGENIZATION OF THE TISSUE OCCURS AT APPRECIABLE TIMES AFTER THE DEATH OF THE ANIMAL. HOWEVER, NEITHER THE SOLUBLE-PARTICULATE DISTRIBUTION NOR THE TOTAL ASSAYABLE ACTIVITY OF RAT BRAIN HEXOKINASE WAS AFFECTED BY DELAY OF HOMOGENIZATION FOR AS LONG AS 90 MIN AFTER DEATH OF THE ANIMAL. THE LATENT RAT BRAIN HEXOKINASE IS, THEREFORE, NOT ANALOGOUS TO THE INACTIVE, PARTICULATE PHOSPHOFRUCTOKINASE OF HEART.

THESE RESULTS HAVE SHOWN THAT APPRECIABLE AMOUNTS OF THE HEXOKINASE IN BRAIN MITOCHONDRIA CAN EXIST IN LATENT FORM. SINCE THE "ASSAYABLE" AND LATENT POOLS OF MITOCHONDRIAL HEXOKINASE APPARENTLY CONTAIN A COMMON ISOZYME, IT SEEMS PROBABLE THAT THE ONLY DIFFERENCE BETWEEN THESE POOLS IS IN THE NATURE OF THEIR MITOCHONDRIAL BINDING SITES. THE SIMPLEST EXPLANATION FOR THE LATENT ACTIVITY IS THAT THE ENZYME IS BOUND AT A SITE INACCESSIBLE TO EXOGENOUS SUBSTRATE. THE CONDITIONS FOUND TO EXPOSE LATENT HEXOKINASE ACTIVITY ALSO RESULT IN EXPOSURE OF LATENT CYTOCHROME OXIDASE OF RAT BRAIN MITOCHONDRIA (KOCH AND LINDALL, 1966), AN ENZYME CONSIDERED TO HAVE AN INTRAMITOCHONDRIAL LOCATION. IF THE INNER MEMBRANE OF BRAIN MITOCHONDRIA, LIKE THAT OF GUINEA PIG LIVER MITOCHONDRIA (ROSE AND WARMS, 1967), CONTAINS HEXOKINASE BINDING SITES, THIS COULD BE THE LOCATION OF THE LATENT ENZYME. THE EXACT LOCATION OF THE HEXOKINASE IN THE MITOCHONDRIA REMAINS A TOPIC FOR FUTURE STUDY. THE VERY LARGE PROPORTION OF THE MITOCHONDRIAL HEXOKINASE THAT CAN BE FOUND IN LATENT FORM SUGGESTS THAT THIS POOL OF HEXOKINASE MAY PLAY AN IMPORTANT PART IN THE PHYSIOLOGICAL FUNCTION OF THE ENZYME.

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REFERENCES

CRANE, R. K., AND SOLS, A., J. BIOL. CHEM., 203, 273 (1953).

HERNANDEZ, A., AND CRANE, R. K., ARCH. BIOCHEM. BIOPHYS., 113, 223 (1966).

JOHNSON, M. K., BIOCHEM. J., 77, 610 (1960).

KATZEN, H. M., AND SCHIMKE, R. T., PROC. NATL. ACAD. SCI. U.S., 54, 1218 (1965).

KOCH, R. B. AND LINDALL, A. W., J. NEUROCHEM., 13, 1231 (1966).

LOWRY, O. H., PASSONNEAU, J. V., HASSELBERGER, F. X., AND SCHULZ, D. W., J. BIOL. CHEM., 239, 18 (1964).

MANSOUR, T. E., WIKID, N., AND SPROUSE, H. M., J. BIOL. CHEM., 241, 1512 (1966).

ROSE, I. A., AND WARMS, J. V. B., J. BIOL. CHEM., 242, 1635 (1967).

SACKTOR, B., WILSON, J. E., AND TIEKERT, C. G., J. BIOL. CHEM., 241, 5071 (1966).

SALTMAN, P., J. BIOL. CHEM., 200, 145 (1953).