Journal of Bioenergetics and Biomembranes, Vol. 24, No. 1, 1992

Minireview

Hexokinase Receptors: Preferential Enzyme Binding in Normal Cells to Nonmitochondrial Sites and in Transformed Cells to Mitochondrial Sites

Krishan K. Arora,[1] David M. Parry,[1,2] and Peter L. Pedersen[1,*]

Received June 18, 1991; revised July 15, 1991

Hexokinase plays an important role in normal glucose-utilizing tissues like brain and kidney, and an even more important role in highly malignant cancer cells where it is markedly overexpressed. In both cell types, normal and transformed, a significant portion of the total hexokinase activity is bound to particulate material that sediments upon differential centrifugation with the crude "mitochondrial" fraction. In the case of brain, particulate binding may constitute most of the total hexokinase activity of the cell, and in highly malignant tumor cells as much as 80 percent of the total. When a variety of techniques are rigorously applied to better define the particulate location of hexokinase within the crude "mitochondrial fraction," a striking difference is observed between the distribution of hexokinase in normal and transformed cells. Significantly, particulate hexokinase found in rat brain, kidney, or liver consistently distributes with nonmitochondrial membrane markers whereas the particulate hexokinase of highly glycolytic hepatoma cells distributes with outer mitochondrial membrane markers. These studies indicate that within normal tissues hexokinase binds preferentially to nonmitochondrial receptor sites but upon transformation of such cells to yield poorly differentiated, highly malignant tumors, the overexpressed enzyme binds preferentially to outer mitochondrial membrane receptors. These studies, taken together with the well-known observation that, once solubilized, the particulate hexokinase from a normal tissue can bind to isolated mitochondria, are consistent with the presence in normal tissues of at least two different types of particulate receptors for hexokinase with different subcellular locations. A model which explains this unique transformation-dependent shift in the intracellular location of hexokinase is proposed.

KEY WORDS: Mitochondria; hexokinase; normal and tumor cells; enzyme localization; subcellular fractionation; receptor for binding; monoamine oxidase; NADPH-cytochrome *c* reductase; transformation.

INTRODUCTION

Hexokinase (ATP: D-hexose 6-phosphotransferase, EC 2.7.1.1) is the first enzyme of the glycolytic reactions which commits glucose to catabolism by catalyzing the reaction

$$\text{Glucose} + \text{MgATP} \rightleftharpoons \text{Glucose-6-P} + \text{MgADP}$$

Previous studies from this and other laboratories have shown that the hexokinase activity in rapidly growing, highly glycolytic tumor cells is markedly elevated (Weber, 1972; Weinhouse, 1972; see also Table I), with 50–80% bound to the mitochondrial fraction (Rose and Warms, 1967, Bustamante and Pedersen, 1977; Bustamante *et al.*, 1981; Parry and

[1]Laboratory For Molecular and Cellular Bioenergetics, Department of Biological Chemistry, The Johns Hopkins University, School of Medicine, Baltimore, Maryland 21205.
[2]Present Address: Department of Clinical Chemistry, St. Boniface Hospital, 409 Tache Ave., Winnipeg, Manitoba R2H 2A6, Canada.
*To whom correspondence should be addressed.

0145-479X/92/0200-0047$06.50/0

Pedersen, 1983). Here it has preferred access to intramitochondrially generated ATP (Arora and Pedersen, 1988) and is also more resistant to glucose-6-P inhibition (Bustamante and Pedersen, 1977). A rigorous subcellular localization study conducted in this laboratory has shown that hexokinase activity in these cells is indeed bound to the outer mitochondrial membrane and not to contaminating membranes (Parry and Pedersen, 1983). In addition, experiments conducted with dicyclohexylcarbodiimide indicate that mitochondrial porin (also known as VDAC) may form a part of the receptor complex for hexokinase binding (Nakashima *et al.*, 1986). Finally, recent cloning and sequencing studies indicate that a hydrophobic set of 12 amino acids at the N-terminus may play a major role in binding tumor hexokinase to this receptor (Polakis and Wilson, 1985; Arora *et al.*, 1990).

In contrast to rapidly growing tumor cells, the hexokinase activity of most normal tissues is quite low. One exception is brain which is a glucose-utilizing tissue (see Table I). Numerous studies conducted over the past 40 years confirm that a significant portion of the hexokinase activity of normal tissues is particulate bound. These tissues include brain, skeletal muscle, mammary gland, kidney, retina, heart, intestine, lung, epididymal fat pad, adrenal medulla, diaphragm, uterus, pancreas, and spleen (see review by Wilson, 1984 and references therein). Although in most of these studies, little or no attempt was made to determine the "particulate" nature of hexokinase, it has been generally assumed that hexokinase is associated with the mitochondria. Perhaps the most compelling piece of evidence taken in support of the view that hexokinase from a normal tissue is bound to mitochondria are studies which show that purified brain hexokinase can bind to isolated liver mitochondria (Felgner *et al.*, 1979). Unfortunately, these studies only show that mitochondria contain potential receptor sites for hexokinase, but do not provide any insight into whether these sites or some other receptor sites are occupied by hexokinase within the tissue of interest, in this case brain.

In this review, we have reevaluated those studies bearing on the subcellular localization of hexokinase of normal tissues, particularly in liver, kidney, and brain where rigorous subfractionation studies have now been performed (Parry and Pedersen, 1983, 1984, 1990). As will be noted below, the available evidence suggests that both nonmitochondrial and mitochondrial receptor sites for hexokinase are available in normal tissues, but that nonmitochondrial receptor sites are preferentially occupied in liver, kidney, and brain. As this is in sharp contrast to what is observed in poorly differentiated, malignant tissues, it is proposed that there may be a transformation-dependent redistribution of hexokinase from nonmitochondrial to mitochondrial receptors.

Table I. Relative Activities of Hexokinase Associated with the "Mitochondrial Fraction" from Hepatomas and a Variety of Normal Tissues[a]

Tissue source[b]	Specific activity (milliunits/mg protein)[c]
Novikoff Hepatoma	1800–1900
AS-30D Hepatoma	1500–1800
Liver	3–8
Heart	38
Skeletal muscle	16–106
Kidney	40–166
Small intestine	54
Brain	495–690

[a] "Mitochondrial fraction" is defined here as that fraction obtained by normal differential centrifugation procedures (see text). Such fractions, although highly enriched in mitochondria, also contain nonmitochondrial particulate material.

[b] For references to tissue sources, see Arora and Pedersen (1988).

[c] One milliunit is defined as the formation of 1 nmol of NADPH per min.

A BRIEF REVIEW OF METHODS USED TO LOCALIZE MITOCHONDRIAL ENZYMES

Various methods which have been employed for subcellular localization of mitochondrial enzymes are as follows:

a. Differential Centrifugation: Many investigators studying properties of mitochondria from various tissues have employed fractions prepared by differential centrifugation of a tissue homogenate. However, mitochondria prepared in this way are not homogeneous and are often contaminated with other organelles or membrane fragments. These include endoplasmic reticulum, lysosomes, peroxisomes and some plasma membrane fragments which co-sediment with the mitochondria. Consequently, these partially purified preparations, containing intact mitochondria, are not recommended for studies in which there may be a question about whether a given process, enzyme, or component macromolecule is localized in mitochondria.

b. Density Gradient Centrifugation: Mitochondria prepared by differential centrifugation can be further purified by density gradient centrifugation involving either a continuous or a discontinuous sucrose gradient; or a continuous Ficoll or Metrizamide gradient (Pedersen *et al.*, 1978). Although, this procedure yields somewhat pure preparations free from lysosomes and peroxisomes, some contaminating microsomes remain.

c. Digitonin Fractionation: Mitochondria prepared by differential and/or density gradient centrifugation are titrated with increasing concentration of digitonin and the release of various marker enzymes is followed. This procedure, which was perfected by Schnaitman and Greenawalt (1968) and used to localize known mitochondrial enzymes, and to distinguish them from contaminating microsomal enzymes, has been used for many years as the "gold standard" for determining whether an enzyme found in the mitochondrial fraction is, in fact, mitochondrial.

d. Density Gradient Centrifugation of Mitochondria following Loading with Barium Phosphate: As indicated earlier, mitochondrial preparations even after density gradient centrifugation are not completely free from contaminating microsomes. Therefore, another way to identify the particulate material with which an enzyme found in a crude mitochondrial fraction is associated, is to load these mitochondria with barium phosphate prior to centrifugation. Barium is taken up on the mitochondrial calcium transporter (Fiskum and Lehninger, 1982) and forms insoluble complexes with phosphate. Therefore, if an enzyme is associated with mitochondria, it will band at a higher density after barium phosphate loading whereas no obvious specific effect of loading should be observed on the distribution of the nonmitochondrial marker enzymes. It remains possible, however, even with this technique that a particularly "sticky" membrane fragment derived from another organelle could adhere to the mitochondria.

e. Electron Microscopy and Immunochemical Methods: The major reason for viewing mitochondria in the electron microscope is to determine whether they are ultrastructurally intact and to estimate the degree of gross contamination by other organelles. When coupled to immunochemical approaches, electron microscopy becomes a more reliable tool for localizing various enzymes and proteins within the subcellular compartments of a cell. Unfortunately, such techniques cannot readily distinguish between an enzyme that is truly bound to an organelle and one that is localized on a closely associated membrane or cytoskeletal component.

The authors of this review emphasize that methods *a* and *b* are not reliable for unequivocally investigating the subcellular localization and distribution of an enzyme, while a systematic study involving methods *c* and *d* coupled with an electron microscopic-immunochemical approach (i.e., method *e*) should provide a fairly accurate picture.

APPLICATION OF THE ABOVE METHODS TO LOCALIZE HEXOKINASE IN NORMAL AND TUMOR CELLS

When Methods *c* and *d* above are rigorously applied to localize particulate hexokinase in normal and tumor cells, it is found that the activity of this critical glycolytic enzyme correlates with microsomal markers in the normal tissues brain, kidney, and liver and with outer mitochondrial membrane markers in tumor cells. These studies (Parry and Pedersen 1983, 1984, 1990) are briefly summarized below, and discussed relative to the work of other investigators.

Digitonin Fractionation

Figure 1 illustrates "digitonin solubilization profiles" of "mitochondrial" fractions from normal and tumor tissues. It is quite evident that such profiles obtained for brain, kidney, and liver are markedly different from those obtained for the hepatoma studied. In all three normal tissues, hexokinase release correlates best with that of NADPH-cytochrome *c* reductase, a microsomal marker (three upper panels), whereas in the hepatoma it releases best with monoamine oxidase, an outer mitochondrial membrane marker (lower panel). These results indicate that the particulate location of hexokinase in hepatoma cells is the outer mitochondrial membrane, whereas in normal cells it is localized within an unidentified microsomal fraction. (Figure 1 indicates that the concentration of digitonin required to solubilize marker enzymes from mitochondria prepared from various tissues is different for each tissue. These observed differences may be due (a) partly to the different mitochondrial protein concentrations employed for digitonin fractionation and (b) partly to the differences in the mitochondrial cholesterol content.)

In brain, hexokinase is highly resistant to digitonin solubilization, even after the outer mitochondrial membrane located enzyme monoamine oxidase has

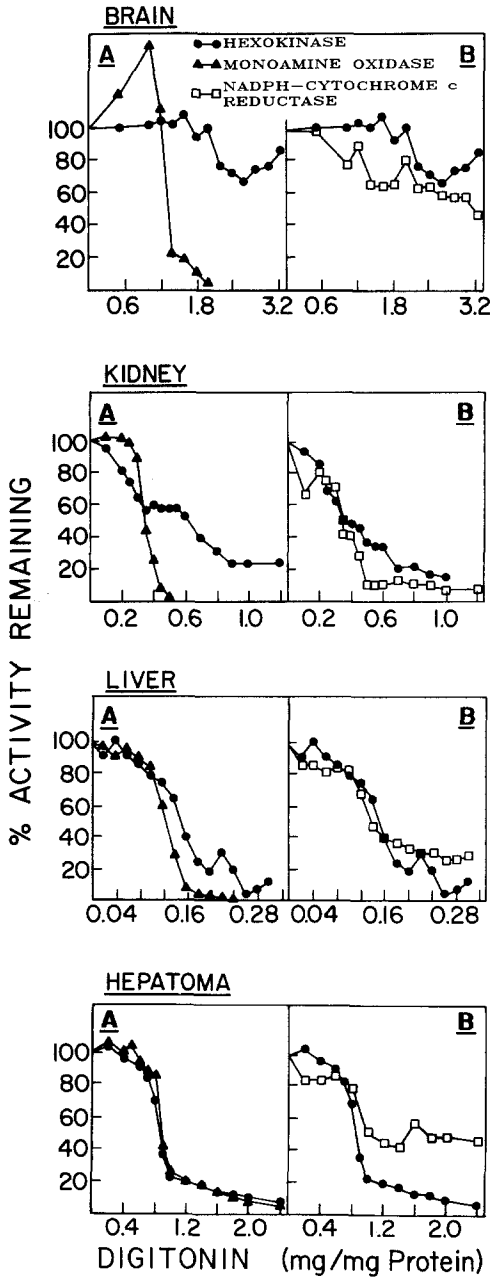

Fig. 1. Hexokinase release following digitonin fractionation of mitochondrial fractions prepared from brain, kidney, liver, and tumor tissues. Mitochondrial preparations were treated with increasing concentrations of digitonin, and the pellets obtained after sedimenting the digitonin-treated mitochondria at 20,000 × *g* for 15 min were assayed for hexokinase (●), monoamine oxidase (▲), and NADPH-cytochrome *c* reductase (□). Results replotted from the data of Parry and Pedersen (1983, 1984, 1990) are expressed as percentage of activity recovered in the pellet obtained from the control. [Permission obtained from the *Journal of Biological Chemistry*.] For details of the experimental procedures for preparing mitochondria and enzyme assays, see Parry and Pedersen (1983, 1984, 1990).

been almost completely released (Fig. 1, panel A for brain). Electron micrographs of the mitochondrial preparation from brain before and after digitonin treatment corroborate these results (Parry and Pedersen, 1990). At high digitonin concentration essentially all structures are severely disrupted leaving membrane fragments which can still be pelleted at 12,000 × *g*. It is these fragments which retain substantial hexokinase activity but essentially no monoamine oxidase activity.

Density Gradient Centrifugation of Mitochondria following Loading with Barium Phosphate

An additional set of experiments performed with barium phosphate loaded mitochondria followed by density gradient centrifugation confirmed mitochondria as the intracellular site of particulate hexokinase in hepatoma cells (Parry and Pedersen, 1983). In these studies, both hexokinase and the mitochondrial marker succinate dehydrogenase were shown to band at a higher density than normal, whereas the distribution of the endoplasmic reticulum marker, NADPH-cytochrome *c* reductase, was not affected. Similar experiments applied to the "mitochondrial" fraction of brain revealed that hexokinase again distributes best with the microsomal fraction (Parry and Pedersen, 1990).

Subcellular localization studies of hexokinase in normal tissues by other investigators have all been interpreted as indicating that the particulate location of hexokinase is mitochondrial, although no comprehensive subcellular fractionation studies by these workers were performed. Surprisingly, previous workers have generally based their assumption of a mitochondrial location for hexokinase in normal tissues on their findings that it sediments upon differential centrifugation with the crude mitochondrial fraction (e.g., Katzen *et al.*, 1970; Ballatori and Cohen, 1981). Undoubtedly, these crude mitochondrial fractions were extensively contaminated by other organelles and membrane fragments therefrom.

It is interesting to compare previous studies on the brain "mitochondrial" fraction (Craven *et al.*, 1969; Dorbani *et al.*, 1987), in which digitonin titration was used also to monitor hexokinase release, with those presented in Fig. 1. Unfortunately, in these earlier studies either only a few concentrations of digitonin were used (Craven *et al.*, 1969) or the release of microsomal markers was not documented (Dorbani *et al.*, 1987). Although hexokinase and porin followed

different release patterns upon titration with digitonin, Dorbani *et al.* concluded that hexokinase is located in brain mitochondria as a porin–hexokinase complex located in a cholesterol-free outer membrane domain together with inner membrane components, i.e., contact sites. This conclusion was not confirmed by conducting experiments using an alternate method to verify whether hexokinase becomes enriched in the heavy mitochondrial fraction after preloading the mitochondria with barium phosphate. Significantly, Parry and Pedersen (1990) in their most recent study show that it does not.

In another study by Kottke *et al.* (1988) designed to localize brain hexokinase, microsomal marker enzyme release was also not monitored. Nevertheless, the conclusion was made that hexokinase resides within the outer mitochondrial compartment, presumably at contact sites between inner and outer mitochondrial membranes. This conclusion is based largely upon an electron microscopic-immunochemical approach. Although elegant, this approach is not able to distinguish between hexokinase bound to mitochondrial and contaminating nonmitochondrial membrane fragments in the putative contact site fractions. More recently, Lynch *et al.* (1991), using only an immunocytochemical approach and three-dimensional confocal microscopy, have analyzed the localization of hexokinase in an astrocyte cell line in culture and claim that nearly 70% of cellular hexokinase is associated with mitochondria. Again, this elegant approach is not able to distinguish hexokinase bound to mitochondria from that bound to some other component closely associated with the mitochondria *in vivo*. Also, artifacts due to the experimental manipulations of paraformaldehyde-fixed astrocytes cannot be ruled out. These authors provide no biochemical evidence, e.g., the relative release of microsomal and mitochondrial marker enzymes, in support of their results. In addition, it is interesting to note that previous studies based on fractionation techniques of astrocytes indicate that a large part of the total hexokinase activity (80%) is soluble and only 20% is found in the particulate fraction containing the mitochondria (Lusk *et al.*, 1980).

Binding of Hexokinase to Rat Liver Mitochondria *In Vitro*

As shown in Table I, the liver mitochondrial fraction contains a very low amount of hexokinase. The release of this particulate hexokinase correlates best with the release of NADPH-cytochrome *c* reductase, a microsomal marker (Fig. 1B). Thus, hexokinase endogenously associated with isolated liver mitochondria appears to be bound to microsomal, rather than mitochondrial, membranes. Conversely, when hexokinase solubilized from hepatoma mitochondria is added to the liver mitochondrial fraction, it readily binds and, in this case, the bound hexokinase release correlates with the release of the outer mitochondrial membrane marker monoamine oxidase (see Fig. 2). This experiment clearly indicates that liver mitochondria do, in fact, contain receptor sites in the outer membrane for hexokinase binding. This *in vitro* binding experiment has been demonstrated also using both solubilized and purified brain hexokinase (Kurokawa *et al.*, 1979; Felgner *et al.*, 1979). In fact, the hexokinase "porin" receptor site was discovered in liver mitochondria by using brain hexokinase (Felgner *et al.*, 1979). These results indicate that brain hexokinase, once solubilized from its microsomal receptor sites in brain tissue, has the capacity to bind to receptor sites in the outer mitochondrial membrane.

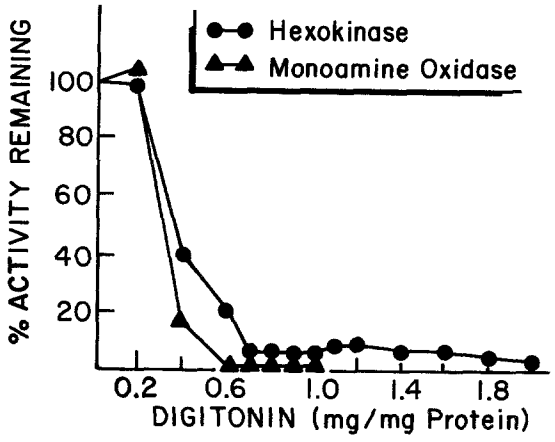

Fig. 2. Release of exogenously bound hexokinase from liver mitochondria by treatment with increasing concentrations of digitonin. Rat liver mitochondria to which solubilized tumor hexokinase had been bound were treated with increasing concentrations of digitonin and the pellets obtained after sedimenting the digitonin-treated mitochondria at 20,000 × *g* for 15 min. Assays for hexokinase (●), and monoamine oxidase (▲) were then performed. Results replotted from the data of Parry and Pedersen (1983) are expressed as percentage of activity recovered in the pellet obtained from the control. [Permission obtained from the *Journal of Biological Chemistry*.] For details of the experimental procedures, see Parry and Pedersen (1983).

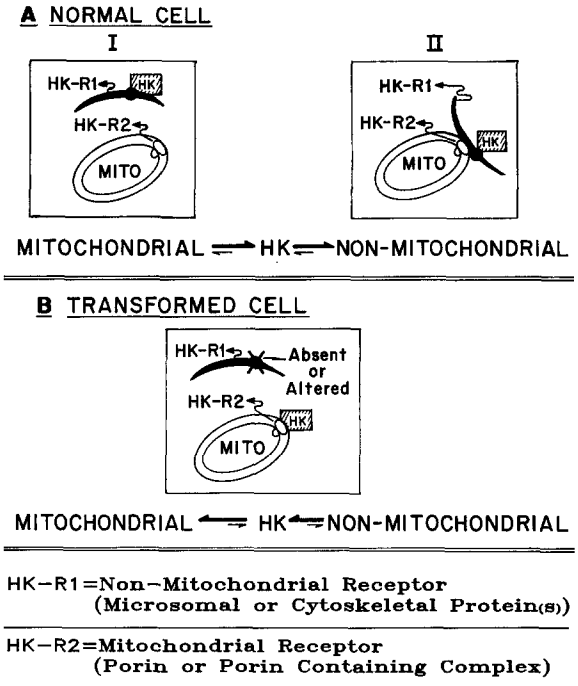

Fig. 3. Models accounting for the differential localization of hexokinase in normal and transformed cells. A: Hexokinase *endogenous to normal cells* is depicted as exhibiting a higher affinity for nonmitochondrial receptors (e.g., on microsomal and/or cytoskeletal elements) than for outer mitochondrial membrane receptors. B: Upon cell transformation, or as a consequence of it, it is suggested that hexokinase moves from its normal "nonmitochondrial" receptors (Panel A) to receptors in the outer mitochondrial membrane (Panel B), presumably porin molecules or porin-containing complexes. Mito, mitochondria; HK, hexokinase.

Differential Localization of Hexokinase in Normal and Transformed Cells

Taken together, the above results from various laboratories strongly indicate that there are two different classes of hexokinase receptors in normal tissues, one of which is associated with the outer mitochondrial membrane, and the other with a nonmitochondrial component (microsomal or membrane cytoskeletal element(s)). As emphasized in the model shown in Fig. 3, the degree to which hexokinase binds to either class of receptors may be governed by its relative affinity for them. It seems clear from the results shown in Fig. 1 that hexokinase, at least in brain and kidney, has a greater affinity for nonmitochondrial receptors than receptors associated with the outer mitochondrial membrane. In highly glycolytic tumor cells where hexokinase subfractionates together with mitochondrial rather than nonmitochondrial components, we propose that either the nonmitochondrial receptor is lost upon transformation (or as a consequence of it), or structurally altered (Fig. 3B). As a consequence, hexokinase in tumor cells preferentially targets mitochondrial receptors.

An alternative possibility is that upon cell transformation (or as a consequence of it), a unique hexokinase isoform is expressed which targets mitochondrial rather than nonmitochondrial receptors. Significantly, most of the amino acid differences observed to date between brain and tumor hexokinases lie in the "hinge" region where the two halves of the molecule, N- and C-terminal, are fused together (Arora *et al.*, 1990). It is possible that these residues distinct to some tumor hexokinases may be involved in targeting these enzymes to mitochondrial receptors. Future experiments using site-directed mutagenesis can be used to test this hypothesis.

With regard to the above two hypotheses concerning the redistribution of hexokinase upon cell transformation, we believe it possible that a similar redistribution may occur upon normal cell dedifferentiation. Thus, we would predict that particulate hexokinase of fetal tissues, in contrast to what is observed for normal tissues (Parry and Pedersen, 1983, 1984, 1990), preferentially binds to mitochondrial rather than to nonmitochondrial receptors.

Finally, it is interesting to inquire about the identity of the cellular component which contains nonmitochondrial hexokinase receptor sites. There are two candidates at this time, the endoplasmic reticulum and the cytoskeletal network. Support for a role for the endoplasmic reticulum is derived both from digitonin fractionation studies (Fig. 1) and from the observation that both hexokinase and endoplasmic reticulum enzymes contain mostly hydrophobic residues at their N-termini (Schwab and Wilson, 1989; Arora *et al.*, 1990; Haugen *et al.*, 1977; Heineman and Ozols, 1984; Black and Coon, 1982). Although, there is no experimental support for a role of the cytoskeletal network in binding hexokinase, there have been several reports demonstrating that most of the glycolytic enzymes of brain and other tissues do interact with actin (Knull, 1978; Clark and Morton, 1982; Walsh and Knull, 1987) and tubulin and microtubules (Walsh *et al.*, 1989), and other reports suggesting that actin interacts with mitochondria (Ball and Finger, 1982). It is also interesting to note the recent results of Wilson and his colleagues (Lachaal *et al.*, 1990) demonstrating the binding of Type I hexokinase to the human erythrocyte glucose transporter *in vitro*. From the above reports, it seems clear that our findings

(Parry and Pedersen, 1983, 1984, 1990) that hexokinases from normal tissues bind to nonmitochondrial receptor sites are not unusual. Finally, a close association with mitochondria, of the nonmitochondrial component (e.g., endoplasmic reticulum or cytoskeletal) containing hexokinase receptors [Figure 3A(II)], may explain the recent results of Beltrandel-Rio and Wilson (1991) on "preferred access" of brain hexokinase to mitochondrially generated ATP.

ACKNOWLEDGMENTS

The authors are grateful for support from NIH Grant CA 32742 (to P.L.P.), and for NIH Fogarty International Fellowship F05 TWO-3082 and Juvenile Diabetes Foundation Fellowship 82F094 to D.M.P.

REFERENCES

Arora, K. K., and Pedersen, P. L. (1988). *J. Biol. Chem.* **263**, 17422–17428.

Arora, K. K., Fanciulli, M., and Pedersen, P. L. (1990). *J. Biol. Chem.* **265**, 6481–6488.

Ball, E. H., and Finger, S. J. (1982). *Proc. Natl. Acad. Sci. USA* **79**, 123–126.

Ballatori, N., and Cohen, J. J. (1981). *Biochim. Biophys. Acta.* **657**, 448–456.

BeltrandelRio, H., and Wilson, J. E. (1991). *Arch. Biochem. Biophys.* **286**, 183–194.

Black, S. D., and Coon, M. J. (1982). *J. Biol. Chem.* **257**, 5929–5938.

Bustamante, E., and Pedersen, P. L. (1977). *Proc. Natl. Acad. Sci. USA* **74**, 3735–3739.

Bustamante, E., Morris, H. P., and Pedersen, P. L. (1981). *J. Biol. Chem.* **256**, 8699–8704.

Clark, F. M., and Morton, D. J. (1982). *Biochem. Biophys. Res. Commun.* **109**, 388–393.

Craven, P. A., Goldblatt, P. J., and Basford, R. E. (1969). *Biochemistry* **8**, 3525–3532.

Dorbani, L., Jancsik, V., Linden, M., Leterrier, J. F., Nelson, B., and Rendon, A. (1987). *Arch. Biochem. Biophys.* **252**, 188–196.

Felgner, P. L., Messer, J. L., and Wilson, J. E. (1979). *J. Biol. Chem.* **254**, 4946–4949.

Fiskum, G., and Lehninger, A. L. (1982). In *Calcium and Cell Function* (Cheung, W. Y., ed.), Vol. 2, Academic Press, New York, pp. 39–80.

Haugen, D. A., Armes, L. G., Yasunobu, K. T., and Coon, M. J. (1977). *Biochem. Biophys. Res. Commun.* **77**, 967–973.

Heinemann, F. S., and Ozols, J. (1984). *J. Biol. Chem.* **259**, 797–804.

Katzen, H. M., Soderman, D. D., and Wiley, C. E. (1970). *J. Biol. Chem.* **245**, 4081–4096.

Kottke, M., Adams, V., Riesinger, I., Bremm, G., Bosch, W., Brdiczka, D., Sandri, G., and Panfili, E. (1988). *Biochim. Biophys. Acta.* **935**, 87–102.

Knull, H. R. (1978). *Biochim. Biophys. Acta.* **522**, 1–9.

Kurokawa, M., Kimura, J., Tokuoka, S., and Ishibashi, S. (1979). *Brain Res.* **175**, 169–173.

Lachaal, M., Wilson, J. E., and Jung, C. Y. (1990). *The FASEB J.* **4(7)**, Abstr. No. 1230.

Lusk, J. A., Manthorpe, C. M., Kao-Jen, J., and Wilson, J. E. (1980). *J. Neurochem.* **34**, 1412–1420.

Lynch, R. M., Fogarty, K. E., and Fay, F. S. (1991). *J. Cell. Biol.* **112**, 385–395.

Nakashima, R. A., Mangan, P. S., Colombini, M., and Pedersen, P. L. (1986). *Biochemistry* **25**, 1015–1021.

Parry, D. M., and Pedersen, P. L. (1983). *J. Biol. Chem.* **258**, 10904–10912.

Parry, D. M., and Pedersen, P. L. (1984). *J. Biol. Chem.* **259**, 8917–8923.

Parry, D. M., and Pedersen, P. L. (1990). *J. Biol. Chem.* **265**, 1059–1066.

Pedersen, P. L., Greenawalt, J. W., Reynafarje, B., Hullihen, J., Decker, G. L., Soper, J. W., and Bustamante, E. (1978). *Methods Cell Biol.* **20**, 411–481.

Polakis, P. G., and Wilson, J. E. (1985). *Arch. Biochem. Biophys.* **236**, 328–337.

Rose, I. A., and Warms, J. V. B. (1967). *J. Biol. Chem.* **242**, 1635–1645.

Schnaitman, C., and Greenawalt, J. W. (1968). *J. Cell Biol.* **38**, 158–175.

Schwab, D. A., and Wilson, J. E. (1989). *Proc. Natl. Acad. Sci. USA* **86**, 2563–2567.

Walsh, J. L., and Knull, H. R. (1987). *Biochim. Biophys. Acta* **952**, 83–91.

Walsh, J. L., Keith, T. J., and Knull, H. R. (1989). *Biochim. Biophys. Acta* **999**, 64–70.

Weber, G. (1972). *Gann Monogr. Cancer Res.* **13**, 47–77.

Weinhouse, S. (1972). *Cancer Res.* **32**, 2007–2016.

Wilson, J. E. (1984). In *Regulation of Carbohydrate Metabolism* (Beitner, R., ed.), Vol. 1, CRC Press, Boca Raton, Florida, pp. 45–85.